



TriStar² LB 942 Modular Multimode Microplate Reader

Operating Manual 56550BA2

Rev. No.: 02, 09/2017



Not for use in in-vitro diagnostic (IVD) procedures.

The information in this guide is subject to change without notice.

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This instrument is not designed or intended for use with installations or equipment in hazardous environments. Servicing of the instrument must only be performed by Berthold Technologies Field Service Engineers or service staff authorized by Berthold Technologies.

Please contact our Service Center at service@berthold.com if you have any operational issues.

Berthold Technologies GmbH & Co. KG

Calmbacher Str. 22 75323 Bad Wildbad, Germany www.berthold-bio.com

Telephone +49 7081 177-0 Fax +49 7081 177-100 bio@berthold.com

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1. Prefatory Comments

1.1 Explanation of LED's and Beeps

LED	Instrument status
lights up green	Instrument OK and connection to PC OK
lights up yellow	Instrument OK, no connection to PC
flashes yellow + 1 short beep	New CAN is installed after power on of instrument
lights up yellow + 1 long beep	CAN correctly installed
lights up red	Shortly after power on of the instrument (during initialization)
flashes red + 2 short beeps	Error after power on of instrument / CAN module not correctly installed

1.2 Operating manual

This Operating Manual is structured as follows:

The manual covers all manipulations in a work flow order starting from installation via regular operation to maintenance.

In each section you are guided through the respective procedures step by step. The steps are consecutively numbered in each section. Explanations on the individual steps are added in small type font.

Explanations on the various types of operations are highlighted specifically.

For your convenience, illustrations are placed directly next to the respective text.

1.3 Typographical conventions

<add formula="">, <ok>, <close></close></ok></add>	Buttons are printed inside angular brackets in bold typeface
Menu File , Open dialog box	Menu titles and dialog boxes are printed in bold type
File Open, Options Read	Menu items are also printed in bold type; menu and submenu item are separated by a vertical line.



2. Safety Instructions

2.1 Safety Instructions





Hot surface: Care while touching the cover or the lamp, they can be hot.

Caution! This sign alerts you to important operating procedures with a potential danger of damaging the equipment and endangering your safety on disobeying. Refer to the user and instrument manuals for precautionary instructions.



This operating manual includes information and warnings that have to be observed by the user in order to ensure safe operation of the instruments.

Please do always act according to the following safety instructions, before as well as during operation of the system! Before set up and operation of the instrument it is necessary to read the instructions below as neither safe operation of the instrument nor safety of the user are guaranteed otherwise. Failure to follow the instructions may invalidate the warranty.

The instrument has been manufactured in accordance with the safety requirements for electrical measuring systems. If the law lays down regulations on the installation and/or operation of sample measuring system, then it is the operator's responsibility to adhere to them.

The manufacturer has done everything possible to guarantee that the equipment functions safely, both electrically and mechanically. The user has to make sure that the instrument will be set up and installed properly to guarantee safe operation.

The instruments are tested by the manufacturer and supplied in a condition that allows safe and reliable operation.

- This equipment must be installed and used in accordance with the manufacturer's recommendations. Installation must be performed by properly trained and authorized personnel.
- □ The instrument may only be operated by personnel who have been trained on the use of the system. It is strongly recommended that all users read this manual prior to use.
- □ Never put parts of your body or other devices into the instrument while the unit is in operation.
- □ Remove the transportation lock before switching on the instrument.
- Use the instrument only for the designated application.
- □ The instrument is designed for indoor use only.
- BERTHOLD TECHNOLOGIES assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instrument.



- □ The user is responsible for connecting the instrument in accordance with the valid regulations for electrical instruments.
- □ Set the instrument up to ensure easy access to the mains switch.
- □ The mains supply voltage fluctuations must not exceed +/-10 % of the nominal voltage. Maximum voltage to be applied is 253 VAC.
- The instruments are designed according to these standards: IEC / EN 610 10-1: 2001 (2ed) CAN / CSA C22.2 No 61010-1-04 UL 61010-1, 2nd Edition
- To disconnect the instrument from power the appliance coupler has to be removed from the mains.
- Do not open any instrument doors as long as the instrument is in operation.
- Service and repair work may be carried out by qualified personnel only.
- The operator may only perform the maintenance work described in this user guide.
- □ There are no exchangeable electrical components in the instrument. In case of malfunction call authorized service personnel.
- Use only parts described in this manual for servicing.
- Disconnect power supply before opening the instrument.
- Pull the power cord to disconnect instrument from power supply.
- Upon removal of the front and top parts of the housing no safety measures are in effect. Be aware of any moving parts. The interior of the instrument may reach temperatures that can cause burns. Some parts of the instrument may remain hot without visual indication for some time after the power has been turned off.
- The electronic unit of the detector generates high voltage. Do not touch it during operation!
- If you can see that the instrument has become unsafe to use, switch it off and disconnect it from power supply.
- If liquid gets inside the instruments, pull the power cord. Clean the unit or have it cleaned by an authorized service center.
- Protect yourself from electrostatic charge, as discharge could damage sensitive instrument parts, especially sensitive parts of the computer and electronics boards.
- When the lid is opened (e.g. filter change) ESD can no longer be guaranteed. To avoid any damages to the electronic parts it is recommended to take precautions (touching the metal case of a safety grounded object, wearing a grounding strap, etc.).
- □ The system always has to be primed with solutions recommended by the kit manufacturer.
- Use only reagents recommended by the kit manufacturer.





- Use reagents only in accordance with the kit manufacturer's instructions.
- Do not use any flammable or explosive solutions or liquids whose mixture is flammable or explosive.
- ❑ Waste (when priming/washing the tubing) always has to be disposed properly: if a waste pump is installed, a bottle has to be connected. If no waste pump is present, a suitable prime plate has to be placed below the injectors during priming/washing.
- Injector solutions may be pumped back only if the appropriate reagent bottle is connected.
- Observe all statutory requirements for handling biological waste, reagents and samples.
- □ The operator is responsible for the use of reagents.
- The units are not for use in in-vitro diagnostic (IVD) procedures.
- □ The instrument should be shipped in its own case. For transport all transportation locks (e.g. for the plate carrier) have to be installed.
- □ For instrument cleaning, please refer to the respective sections in this manual.
- Reliable instrument function can be guaranteed only when original spare parts are used.

The tests and service work recommended by the manufacturer has to be performed to make sure that the operator remains safe and that the instrument continues to work correctly. Any service and maintenance work not described in this user guide has to be performed by authorized service personnel. Use the instruments only for the designated application.



2.2 Consignes de Sécurité



Surface chaude: Attention en touchant la couverture ou la lampe – danger de se brûler!

Attention! Ce symbole d'alarme, vous avertit de prêter attention aux consignes opératoires. En effet si vous ne suivez pas ces instructions, il peu y avoir un risque d'endommagement du matériel et également vous faire ecourir des risques pour votre propre sécurité. Il est impératif de respecter les instructions du mode d'emploi et de les respecter.



Ce mode d'emploi contient des informations et avertissements qui doivent être suivis par l'utilisateur afin de garantir un fonctionnement sûr des instruments.



Il est impératif de respecter les consignes de sécurité suivantes non seulement avant la mise en service mais aussi pendant le fonctionnement de l'appareil! Avant l'installation et mise en service de l'instrument tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions de service, autrement ni le fonctionnement correct de l'appareil ni la sécurité de l'utilisateur peuvent être garantis. Ne pas suivre ces instructions de service peut invalider la garantie.

Le appareil a été fabriqué conformément aux prescriptions de sécurité en vigueur pour les appareils de mesure électrique. Si l'installation et/ou l'utilisation des appareils de mesure de prélèvements-échantillons sont/est soumise(s) à des réglementations prescrites par la loi, il appartient à l'utilisateur de les respecter.

Le constructeur a fait tout le nécessaire pour assurer le fonctionnement sûr des appareils (du point de vue électrique, électronique et mécanique). L'utilisateur est tenu de veiller à ce que les appareils soient installés correctement afin d'éviter toute altération de leur utilisation sûre.

Les appareils sont contrôlés à l'usine et livrés dans un état assurant la sécurité de fonctionnement.



Les appareils doivent être mis en service et utilisés strictement conformément aux recommandations du constructeur. La mise en service est réservée au personnel formé et autorisé.

- Les appareils ne doivent être utilisés que par des personnes autorisées et leur utilisation est réservée au personnel compétent. Tous les utilisateurs des appareils sont tenus de lire d'abord ces instructions de service.
- □ Ne mettez jamais des parties de votre corps ou des objets dans l'appareil lorsque celui-ci est en fonctionnement.
- Enlevez le verrouillage de transport avant la mise sous tension de l'appareil.



- Utilisez l'instrument uniquement pour les applications désignées compatibles.
- L'appareil est destiné uniquement pour une utilisation en intérieur de bâtiments.
- BERTHOLD TECHNOLOGIES décline toute responsabilité de dommages résultant d'une utilisation non conforme à l'emploi prévu, y compris les dommages causés à des tiers.
- □ Les variations sur la tension du secteur ne doivent pas dépasser +/-10% de la valeur nominale (max. 253 VAC).
- □ L'utilisateur porte la responsabilité de la mise en service de l'appareil selon les prescriptions électriques en vigueur.
- Les appareils correspondent aux prescriptions de la norme: IEC / EN 610 10-1: 2001 (2ed) CAN / CSA C22.2 No 61010-1-04 UL 61010-1, 2nd Edition
- Pour arrêter et débrancher l'instrument la fiche doit être retirée hors de la prise.
- Ne pas ouvrir le couvercle lors du fonctionnement de l'appareil. Arrêtez l'instrument avant.
- □ Les travaux d'entretien et de réparation devront être confiés exclusivement à des spécialistes dûment formés.
- □ Les travaux d'entretien uniquement décrits dans le manuel peuvent être effectués par l'utilisateur.
- Pour les travaux d'entretien, utiliser exclusivement les pièces mentionnées.
- Avant d'ouvrir l'appareil, couper l'alimentation en courant.
- Arrêter l'appareil avant de retirer la fiche.
- Si vous ouvrez l'appareil, les sécurités ne sont plus activées (capôt et parties de la façade de l'appareil). Faites attention aux parties mobiles. L'intérieur de l'appareil et certaines pièces peuvent atteindre des températures pouvant provoquer des brûlures si il y a contact. Appareil éteint, des parties peuvent rester chaudes alors qu'il n'y a pas d'indication visible de température élevée.
- Positionner l'appareil de manières à ce que les interrupteurs soient accessibles.
- Si vous vous apercevez que le fonctionnement de l'appareil n'est plus sûr, il faut alors l'arrêter et le débrancher de la prise secteur.
- Si du liquide a pénétré dans l'appareil il faut immédiatement le débrancher. Ouvrir l'appareil et le nettoyer ou bien le faire nettoyer par une agence de service après vente autorisée.
- Protégez vous des charges électrostatiques afin d'éviter de provoquer des décharges qui pourraient endommager des parties sensibles de l'appareil telles que les cartes électroniques ou PC.
- Ne pas utiliser des liquides inflammables ou explosifs ni de liquides dont le mélange est inflammable ou explosif.





- Respecter toutes les prescriptions légales concernant la manipulation des déchets biologiques, des réactifs et des échantillons.
- L'utilisateur assume la responsabilité exclusive de l'utilisation des réactifs.
- □ Le dispositif n'est pas destiné à être utilisé dans In Vitro Diagnostic.
- Transporter l'appareil uniquement dans son emballage d'origine. Lors du transport, bloquer le support de plaques à l'aide de la vis d'arrêt.
- Pour le nettoyage de l'instrument veuillez vous référer au paragraphe correspondant dans ce mode d'emploi.
- □ Le fonctionnement correcte ne peut être garantit qu'à la condition que des pièces de rechange appropriées sont utilisées.

Afin d'assurer la sécurité de l'utilisateur et le bon fonctionnement des appareils, effectuer les travaux d'inspection et d'entretien recommandés par le fabricant. Toutes les mesures d'entretien et de réparation allant audelà de celles spécifiées dans ce manuel sont réservées aux techniciens autorisés.



2.3 Sicherheitshinweise



Heiße Oberfläche: Vorsicht beim Berühren der Abdeckung bzw. der Lampe, sie können heiß sein.

Die vorliegende Bedienungsanweisung enthält Informationen und Warnungen, die vom Benutzer befolgt werden müssen, um einen sicheren Betrieb der Geräte zu ermöglichen.

Dieses Zeichen weist den Benutzer auf wichtige Punkte hin, deren Beachtung unerlässlich ist.

Die folgenden Sicherheitshinweise sind sowohl vor der Inbetriebnahme als auch während des Betriebs des Gerätes unbedingt zu beachten. Vor Inbetriebnahme des Gerätes ist es zwingend erforderlich, die Bedienungsanleitung zu lesen, da ansonsten die Sicherheit des Gerätes und des Benutzers nicht gewährleistet wird.

Das Gerät wurde in Übereinstimmung mit den Sicherheitsanforderungen für elektrische Messgeräte hergestellt. Bestehen für die Errichtung und/oder den Betrieb von Probenmessgeräten gesetzlich vorgeschriebene Regelungen, so ist es die Aufgabe des Errichters und Betreibers, diese einzuhalten.

Der Hersteller hat alles unternommen, um ein sicheres Arbeiten der Geräte (bezüglich Elektrik, Elektronik und Mechanik) zu gewährleisten. Der Benutzer muss dafür sorgen, dass die Geräte so aufgestellt und installiert werden, dass ihr sicherer Gebrauch nicht beeinträchtigt wird.

Die Geräte sind werkgeprüft und wurden in betriebssicherem Zustand ausgeliefert.

- Die Geräte dürfen nur von autorisierten Personen in Betrieb genommen und nur von eingewiesenem Personal bedient werden. Alle Benutzer, die mit den Geräten arbeiten, müssen zuerst diese Bedienungsanleitung lesen.
- Die Geräte dürfen nur von dafür geschultem Personal betrieben werden. Es wird allen Anwendern empfohlen, diese Bedienungsanleitung vor Benutzung zu lesen.
- Während des Betriebes nicht in die Geräte faseen oder andere Teile in die Geräte einführen.
- Transportsicherungen vor dem Einschalten entfernen.
- Die Geräte dürfen nur für den vorgesehenen Zweck eingesetzt werden.
- Berthold Technologies übernimmt keinerlei Gewährleistung, auch für Schäden gegenüber Dritten, die durch unsachgemäße Handhabung der Geräte hervorgerufen werden.
- Die Geräte dürfen nur innerhalb geschossenen Räumen betrieben werden.
- □ Die Stromversorgung darf nicht mehr als ±10 % des Nominalwertes aufweisen. Maximal sind 253 V Wechselstrom erlaubt.
- □ Es liegt im Verantwortungsbereich des Anwenders, dass die Geräte nach den lokalen elektrischen Vorschriften installiert werden.



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- Die Geräte entsprechen den Vorschriften der: IEC / EN 610 10-1: 2001 (2ed) CAN / CSA C22.2 No 61010-1-04 UL 61010-1, 2nd Edition
- □ Nicht öffnen, wenn das Gerät in Betrieb ist.
- Service- und Reparaturarbeiten d
 ürfen nur von Fachleuten ausgef
 ührt werden.
- Es dürfen nur die im Handbuch beschriebenen Wartungsarbeiten vom Anwender ausgeführt werden.
- Bei Wartungsarbeiten dürfen nur die angegebenen Teile verwendet werden.
- U Vor dem Öffnen des Gerätes ist die Stromzufuhr zu unterbrechen.
- Um das Gerät vollkommen vom Netz zu trennen, kann das Netzkabel gezogen werden.
- Wenn das Gerät geöffnet ist sind Sicherheitsmaßnahmen nicht mehr in Betrieb. Auf bewegliche Komponenten achten! Das Innere der Geräte kann Temperaturen erreichen, die Verbrennungen verursachen können. Einige Teile können heiß bleiben ohne sichtbare Zeichen, auch nachdem das Gerät abgeschaltet worden ist.
- An der Multiplier-Einheit liegt Hochspannung an. Nicht berühren, wenn das Gerät läuft.
- □ Stellen Sie das Gerät so auf, dass Sie es leicht ein- und ausschalten können.
- Bei Beeinträchtigung der Betriebssicherheit sind die Geräte abzuschalten und vom Netz zu trennen.
- Ist Flüssigkeit in das Innere des Gerätes gelangt, Netzstecker ziehen. Das Gerät öffnen und reinigen bzw. durch eine autorisierte Servicestelle reinigen lassen.
- Elektrostatische Aufladungen (z.B. durch Teppichböden) müssen beim Öffnen des Gerätes verhindert werden, da Entladungen am Gerät zur Beschädigung elektronischer Teile führen können.
- Das System muss immer ausreichend mit den vom Reagenzienhersteller empfohlenen Lösungen gespült werden.
- Es dürfen nur Reagenzien verwendet werden, die vom Reagenzhersteller empfohlen werden.
- □ Reagenzien dürfen nur in der vom Reagenzienhersteller vorgeschriebenen Art und Weise verwendet werden.



Es dürfen keine entzündlichen oder explosiven Flüssigkeiten oder keine Flüssigkeiten, deren Mischung entzündlich oder explosiv ist, verwendet werden.

Es ist immer auf eine korrekte Entsorgung des Abfalls (beim Füllen/Spülen der Leitungen zu achten: Bei integrierter Abfallpumpe ist ein Behälter anzuschließen. Wenn keine Abfallpumpe vorhanden ist, muss beim Spülen/Füllen der Leitungen eine entsprechende Auffangwanne unter den Injektoren plaziert sein.



- Das Zurückpumpen der Injektorflüssigkeit darf nur dann vorgenommen werden, wenn die entsprechenden Reagenzbehälter angeschlossen sind.
- Beachten Sie alle gesetzlichen Vorschriften f
 ür den Umgang mit biologischem Abfall, Reagenzien und Proben.
- Die Anwendung der Reagenzien liegt im alleinigen Verantwortungsbereich des Benutzers.
- Die Geräte sind nicht f
 ür den Einsatz in der In Vitro Diagnostik bestimmt.
- Das Gerät sollte nur in der eigenen Verpackung transportiert werden. Beim Transport ist darauf zu achten, dass alle Transportsicherungen eingesetzt werden (z.B. die Sicherung für den Plattenträger).
- Zum Reinigen des Gerätes bitte den entsprechenden Teil dieser Bedienungsanleitungen beachten.
- Ordungsgemäße Funktionalität kann nur bei Verwendung der Originalersatzteile garantiert werden.

Für die Sicherheit des Benutzers und die Funktionsfähigkeit der Geräte sind die vom Hersteller empfohlenen Überprüfungen und Wartungsmaßnahmen durchzuführen. Alle über die Betriebsanleitung hinausgehenden Wartungsund Instandhaltungsmaßnahmen dürfen nur von autorisierten Technikern ausgeführt werden.



3. Warranty and Technical Issues

3.1 Special spare parts

The following spare parts are safety parts: Use the original part from the manufacturer or direct agent only.

Power supply	input 100 - 240 VAC, 4 A output 24 VDC, 9.2 A, max 221 W	GST220A24-R7B part no. 59048

3.2 Warranty statement

The instrument is sold in accordance with the general conditions of sale of Berthold Technologies GmbH & Co KG and its affiliates and representatives.

Berthold Technologies warrants this product to be free of defects in material and workmanship for a period of 12 months from the date of delivery, ex works Bad Wildbad.

Berthold Technologies or its authorized representative will repair or replace, at its option and free of charge, any product that under proper and normal use proves to be defective during the warranty period.

Berthold Technologies shall in no event be liable or responsible for any incidental or consequential damage, either direct or indirect.

The above warranty shall not apply if:

- a) the product has not been operated in accordance with the operating manual
- b) the product has not been regularly and correctly maintained
- c) the product has not been repaired or modified by a Berthold Technologies authorized representative or user
- d) parts other than original Berthold Technologies parts are used
- e) the product and parts thereof have been altered without written authorization from Berthold Technologies GmbH & Co KG
- e) the product has not been returned properly packed in the original Berthold Technologies packaging

This warranty does not apply to any third party product involved in the application.

Berthold Technologies reserves the right to refuse to accept the return of any product that has been used with radioactive or (micro)biological substances, or any other material that may be deemed hazardous to employees of Berthold Technologies. Such products have to be properly decontaminated and marked. Before returning products to Berthold Technologies ensure the devices are properly decontaminated and the form "**Confirmation on decontamination**" is properly filled in and will be accompanying the product. (See appendix for a blank form)

Before returning products to Berthold Technologies, a returns/repair number must be obtained and clearly identified on the packing and documents. Call Berthold Technolo-



gies to get this number. Retain the original packaging for use if the instrument needs to be returned to Berthold Technologies.

3.3 Customer service

Customer service will be provided in the first instance by the network of Berthold Technologies representatives. In the event of any problem experienced with your instrument, the first recourse should be **your local Berthold Technologies representative**. For further problems requiring hardware or software expertise, the Technical Support group at Berthold Technologies GmbH & Co KG will be available by phone, fax or email to deal with your queries. Here is their address, phone, fax and e-mail:

Berthold Technologies GmbH & Co KG Technical Support Calmbacher Str. 22 75323 Bad Wildbad Germany Phone: +49 7081 177 114 Fax: +49 7081 177 301 Email: <u>service@berthold.com</u>

At the end of this manual you will find a Customer Reply Form (Appendix section). If a problem arises with the instrument which you are not able to resolve, please fill in this form. This form should then be transmitted to your Berthold Technologies representative or to Technical Support at Berthold Technologies, where it will receive early attention.

Please also make sure that you have the relevant information available before contacting Berthold Technologies. Helpful information would include:

- serial numbers, part number, revision: see production label on instrument
- software and firmware versions
- monitor and log files (refer to the respective service manuals)



4. Introduction

4.1 Intended use

The **TriStar²** is a modular multi-technology microplate reader for different types of fluorescent, luminescent and absorbance applications.

The units are not for use in in-vitro diagnostic (IVD) procedures. These units are not designed for use in hazardous areas.

4.2 Description

The **TriStar**² microplate reader is distinguished by its exceptionally high sensitivity allowing detection limits in scientifically relevant magnitudes with low reagent consumption.

Detector sensitivity and stability are the result of Berthold Technologies' experience with thousands of photon counters. **True photon counting** has the benefit that no user parameters need to be set, ensuring the same conditions are used for every measurement during the instrument's entire life time. The fast photon counting circuitry provides a dynamic range in excess of six orders of magnitude, which complements the range of the latest assays.

A proprietary design of the optical system achieves absolute minimisation of cross-talk down to 10⁻⁶ (depending on the type of microplate).

The PC based **ICE operating software** has been designed with the basic research scientist in mind and offers straight forward access to the respective parameter settings. Besides raw data measurements the user can select dual measurements with ratio calculation or kinetic and scanning.

The instrument can read solid plates as well as strip plates from 6 to 384 well formats with a height **not** exceeding 21 mm (respective adapter frames need to be applied).

4.3 Recommendations for proper handling

To obtain good and consistent results please follow these recommendations :

- Do not expose instrument to direct sunlight
- Set up instrument in dry rooms
- Open lid for loading filter/microplates or cleaning only to keep light and dust out
- Keep plate carrier free from dirt
- Remove spilled reagents immediately with damp cloth or optical grade tissue
- Very bright samples may cause saturation of the PMT (indicated by an "Overload" message); let the PMT recover for a few seconds

To avoid damages to mechanical, electrical and optical components obey to these rules:

- Load microplates correctly
- Do not use microplates or strip plates with heights exceeding 21 mm
- Do not fill the microplates above their specified maximum volume
- Do not shake completely filled microplates in the instrument



 Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system; take special care when ice in the trough starts to melt



5. Installation

Read this part completely prior to starting with the first steps and make sure that all prerequisites are met as described below.

5.1 Unpacking and Set up

- 1. Unpack TriStar² and accessories
- 2. Put TriStar² onto an appropriate laboratory desk
- 3. Remove external power supply from its box and connect to power cord



4. Open the big front flap and **remove transportation safety device**



5. Connect USB cable to USB port of instrument





Operating Manual

6. Connect power cord to the respective socket of the instrument



7. Verify the mains switch is in OFF position



Connect instrument only if it is matching!

- 9. Put the jack of the external power supply into the wall outlet
- 10. For the consecutive software installation the instrument should remain **turned off**.



Mains switch Mains socket



5.2 Software Installation

The instrument can be run with either ICE or Mikrowin software. Dependent on your software configuration follow either the instructions for ICE software or Mikrowin software installation respectively.

5.2.1 Installation of ICE operating software

- **Note:** The software requires a computer with Windows operating system (Windows 2000, Windows XP, Windows Vista, Windows 7). For installation local administrator level is recommended but not necessary.
- **Note:** As the software requires some additional resources for proper operation the set up wizard will check for the presence of these resources (.NET Framework 2.0 and Crystal Reports for .NET Framework) on the computer. If the resources are found the installation of Instrument Control and Evaluation (ICE) software is started.

In case these resources are not available on the computer the set up wizard will start with the installation of these resources.

ISSetupPrerequisites

0x0409.ini

JCE.msi

Manual

Autorun

0x0804.ini

- 1. Close all Windows applications before you start installing the software
- 2. **Insert software CD** into CD drive The set up routine starts automatically

In case the installation does not start automatically browse to the CD's root directory and double click **Setup.exe**

3. Click **<Run>** when the Security Warning dialogue appears

This or similar dialogues may appear during consecutive steps of the installation due to Windows security settings. Always confirm the messages to continue the installation.







题 - 日 0

USB WHQL Berthold 2.06.00

1033.ms

Data1.cab

Setup

6. Ir	nstallShield will configure itself	InstallShield Wizard	Preparing to Install ICE Setup is preparing the InstallShield Wizard, which will guide you through the program setup process. Please wait. Configuring Windows Installer
7. C	Click <next></next> to start installation procedure	ICE - InstallShield Wizard	Welcome to the InstallShield Wizard for ICE The InstallShield(R) Wizard will install ICE on your computer. To continue, click Next. WARNING: This program is protected by copyright law and international treaties.
8. C L th 9. C	Choose an installation directory Oue to support issues it is recommended to use he defaulted one Click <next></next>	ICE - InstallShield Wizard Destination Folder Click Next to install to this fold Install ICE to: C:\ICE\	ier, or click Change to install to a different folder. Change

InstallShield



< Back Next > Cancel





Operating Manual

12. Click <finish></finish>	J브 ICE - InstallShield Wizard
	InstallShield Wizard Completed The InstallShield Wizard has successfully installed ICE. Click Finish to exit the wizard.
	< <u>B</u> ack Finish Cancel
 After a successful installation an ICE icon will be visible on the desktop 	

5.2.2 Installation of Mikrowin 2000 operating software

- **Note:** The software requires a computer with Windows operating system (Windows 2000, Windows XP, Windows Vista, Windows 7) . For installation local administrator level is recommended but not necessary.
- **Note:** For the installation of MikroWin and driver software as well as for any updates and upgrades of the respective software the user has to have *local Administrator rights* for the computer.
- **Note:** *Advanced* versions are delivered with a hard lock (parallel or USB) for copy protection. The hard lock is matched with the installation CD. The hardlock needs to be attached during all operations with MikroWin. The *Lite* version needs to get activated with an activation code during or after installation.
- **Note:** When a **USB hard lock** is used the installation has to be performed without the hard lock plugged in. The USB hard lock has to be put into the PC right after installation.
- Close all Windows applications before you start installing the software
 Insert software CD into CD drive The set up routine starts automatically
 In case the installation does not start automatically browse to the CD's root directory and double click Setup.exe



Operating Manual

- 3. Select language and confirm with **<OK>**. The setup assistant is started
- 4. Enter name and company and click <Next>
- 5. Choose **destination location** (see screen shot to the right).

The following path is defaulted C:\Program Files\Mikrowin 2000

For support reasons it is recommended to keep the default settings

If you wish to install the program to another folder, click **<Browse>** and select another folder

6. Click <Next>

Toriginal anial State for 2000 in the Salaring Links
T i unstal la frui locke, deile Host. Tornatal to a different focke, disk direven and paleut worthe locke
Constant fulle Constant fulle

7. Select the setup type

We recommend that you choose **Typical** for your first installation to ensure that all program components are installed.

If you are familiar with the system, you may choose **Custom** to select the components you need for your application. You may especially <u>not</u> want to install the instrument drivers LB96V and Null Device.

- 8. Click **<Next>**
- 9. Select the desired components or deselect those components you don't want to install.
- 10. Click <Next>







× 11. Add program icon to the Mikrowin 2000 program Select Program Folder folder. Setup will add program icons to the Program Folder listed below. You may type a new folder name, or select one from the existing Folders list. Click Next to continue 12. Click <Next>. Program Folders: Installation is carried out and successful comple-MikroWin 2000 tion is indicated. Existing Folders: Adobe Acrobat 4. Autostart Brandstetter . Brother HL-1650_1670N Series Caere-Anwendungen Creative Hewlett-Packard HP PhotoSmart MGI SoftDVD MAX -Microsoft Office Tools < Back Next> Cancel 11. Click <Finish> to complete setup 12. Attach Mikrowin 2000 USB hard lock for Advanced versions or Run the Activation procedure for Lite versions

5.2.3 Activation of MikroWin Lite Software

The Activation prodedure needs to be executed only when a new installation of Mikrowin 2000 has been performed.

 This dialog will be displayed when starting a not yet activated MikroWin 2000 Lite software with- out the instrument switched on (v 4.29 and high- er) It is recommended to switch off and disconnect the instrument during software activation. 	MikroWin 2000 Startup Info Dear valued customer, This is a not yet activated version of MikroWin 2000. Only a successfully activated version of MikroWin 2000 permits you to use the program without limitations. In order to activate your product, please use the Program Activation dialog located in submenu Help of the main menu. This dialog will provide you with the necessary information to activate your copy of MikroWin 2000. Don't show this message again.
2. Go to Help Program Activation	Program Activation
There are 3 ways to acquire the activation code: I) on line via internet (proceed with step 3) II) via email (proceed with step 8) III) via fax (proceed with step 16)	In order to achieve MArcWin 2000 for this computes submit the Program ID Code shown below to us. There are three possibilities to send the Program ID Code. If you computer is connected to the Internet please press the "Julo Activation" button in order to start the advanted program Activation for the Julo activation of the internet please press the "Julo Activation" button in order you have access to the Internet please and an eMail to actiGmarketek de including the Program ID Code view must be "Sand Activation" button is cardiar view of a weather the received Code into the Program Activation for lacence. If you have no access to the Internet please send us a fax including the Program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet you Activation Code via the. These activation Code via the Alles access to the Internet please send us a fax including the Program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet you Activation Code via the Anles access the Internet please you Activation Code via the Anles access to the Internet please send us a fax including the Program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet on the Activation the fax form the program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet on the Activation the fax form the program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet on the Activation the fax form the program ID Code. Press "Show Pac Form" to view an appropriate fax form. After you have submitted your If internet on the Activation Code into the Paces access to the Internet please the Activation Code into
Activation via internet:	Device Data
3. Enter serial number of instrument	Please enter the serial number of the measuremnt device controlled by this program :

Program ID Code :

Activation Code :

6E618 05713 24A20 616E2 6105C 72241

- 4. Click <**Auto Activation**>
- 5. Click <OK> on the next screen displayed to con-



Copy to Clipboard Paste from Clipboard

firm the activation process	
Code will be transferred online and will be auto- matically entered into the respective boxes	
Activation code will be returned within German office hours only	
 Once code is entered in respective fields click <ok></ok> 	
Activation via email:	Profil auswählen
Click <copy clipboard="" to=""></copy>	Profilname: Neu
9. Click < Send eMail >	
10. Select suitable email profile	OK Abbrechen Hife Ontionen >>1
 use "MikroWin Program Activation" as subject and provide these details of your system: Pro- gram ID Code, Device Serial Number and Program Licence Code 	
12. Email with respective activation code will be re- turned within 24 h	
13. Copy code to clipboard.	
14. Re-access the Program activation menu and click < Paste from Clipboard >	
15. Click < OK >	
Activation via fax:	MikroWin 2000 Activation Fax Form
16. Click < Copy to Clipboard >.	To print this order form, dick on Print command in the File pull-down menu.
17. Click < Show Fax Form >.	Fill out the required form fields and enter your Program ID Code. Next, please fax this document to Mikrotek (++49 2204 75071).
18. Paste Program ID Code into respective fields	rod win receive your personal Acavation code wronn are next? days.
and enter additional required information.	Mikrotek Laborsysteme GmbH Telefon: (49)2204 / 74675
	Olper Straße 35 Fax: (49)2204 / 75071 D-51491 Overath , E-Mail: info@mikrotek.de
	Germany Internet: http://mikrotek.de
	I wish to activate my licence of MikroWin 2000.
	Name required
	Address required
	Phone
	Fax required
	Serial Number of the measurement device is:
	My Program ID Code is:
	My Program ID Code is:

5.2.4 Installation of TriStar² driver



In order to be able to communicate with the instrument via the USB port (executing operations and receiving data) the driver software needs to be installed and set up.

The instrument needs to be **switched off** during this process.

- 1. Close all Windows applications before you start installing the software
- 2. Make sure the instrument's power switch is in **OFF** position
- 3. **Insert software CD** into CD drive The set up routine starts automatically

In case the installation does not start automatically browse to the CD's root directory and double click **Setup.exe**

Click **<Yes>** or **<Accept>** or **<Run>** should any warning message appear on your screen

4. Install Shield will prepare the installation



TriStar2 - InstallShield Wizard	
Preparing Setup Please wait while the InstallSh	ield Wizard prepares the setup.
TriStar2 Setup is preparing the setup process. Please wait.	InstallShield Wizard, which will guide you through the rest of the
InstallShield	
	Cancel
TriStar2 - InstallShield Wizard	
motarz - motanomeru wizaru	
	Welcome to the InstallShield Wizard for TriStar2
	Welcome to the InstallShield Wizard for TriStar2 The InstallShield Wizard will install TriStar2 on your computer. To continue, click Next.
	Welcome to the InstallShield Wizard for TriStar2 The InstallShield Wizard will install TriStar2 on your computer. To continue, click Next.
	Welcome to the InstallShield Wizard for TriStar2 The InstallShield Wizard will install TriStar2 on your computer. To continue, click Next.

5. Click <Next>



 Select the installation of MikroWin depending software you are using Click <next></next> 	type for or ICE (or on which kind of evaluation g and have installed prior)	TriStar2 - InstallShield Wizard Options Choose one of the following options. MikroWin Installation ICE Installation InstallShield	<back next=""> Cancel</back>
 Choose an installatio Due to support issues the defaulted one 	n directory it is recommended to use	TriStar2 - InstallShield Wizard Choose Destination Location Select folder where setup will install files. Install TriStar2 to: C:NCE	Change
9. Click <next></next>		InstallShield	< Back Next> Cancel
10. Click <install></install>		TriStar2 - InstallShield Wizard Ready to Install the Program	
Click <accept></accept> or <r< b=""> messages appear</r<>	un> should any warning	The wizard is ready to begin installation. Click Install to begin the installation. If you want to review or change any of your the wizard.	installation settings, click Back. Click Cancel to exit







13 Click - Finish	Device Driver Installation Wizard
	Concerning the Device Driver Installation Wizard The drivers were successfully installed on this computer. To can now connect your device to this computer. If your device came with instructions, please read them first. Driver Name Status Y FTDI CDM Driver Packa Ready to use Y FTDI CDM Driver Packa Ready to use Y FTDI CDM Driver Packa Ready to use
14. Click <finish></finish>	TriStar2 - InstallShield Wizard InstallShield Wizard Complete The InstallShield Wizard has successfully installed TriStar2. Cick Finish to exit the wizard.
15. Connect the USB cable to a USB port of the computer	
16. A message will be shown in the task bar during the USB driver installation	Installing device driver software X Click here for status. Desktop DE
17. After a few minutes a message confirming the successful installation will be displayed in the task bar	Berthold Technologies TriStar 🄌 🗴 Device driver software installed successfully.
 Turn instrument on by putting mains switch into ON position 	



19. Open **ICE** software or **Mikrowin** software dependent on which kind of installation you have done prior





20. Select TriStar² in View menu (ICE)

or

Select the menu item **Installation | Driver** (*Mikrowin*) to open the Installation Driver dialog box with a separate tab for each driver type.

File	liew Instrument Tool	Help				
	Navigation Panel					_
Defau	/ TriStar2	ile Nar	ne	Star	t Measurem	en
	Protocol Wizard	s				
-	Data		2	3	4	
Proto	Result SampleId Standards	nple	Sample	Sample	Sample	
	Result Mode >	ample	Sample	Sample	Sample	

21. Go to Instrument menu and select Properties (ICE)

or

Highlight BertholdTech TriStar2 and click on <Driver Setup> (*Mikrowin*)

nstrument Tools I	Help		
Properties			
Load Plate Unload Plate		Start Measurement	
Injector Settings Prime Wash	2	3	4
Refresh	Sample	Sample	Sample
Excitation Filter Slide	le Sample	Sample	Sample
Boot Instrument	Sample	Sample	Sample
	Instrument Tools I Properties Load Plate Unload Plate Injector Settings Prime Wash Refresh Unload Injector Excitation Filter Slide Plate Editor Boot Instrument	instrument Tools Help Properties Load Plate Unload Plate Injector Settings Prime 2 Wash Refresh Sample Unload Injector Excitation Filter Slide Emission Filter Slide Plate Editor Boot Instrument Sample	Instrument Tools Help Properties Load Plate Unload Plate Injector Settings Prime Wash Refresh Unload Injector Excitation Filter Slide Emission Filter Slide Plate Editor Boot Instrument Sample


- 22. Select the entry starting with **BT60....** (e.g. X BertholdTech TriStar2, Version 0.01 BT601004) in the ComPort section ComPort 0K The raw data are usually displayed as RLU rep-COM1 -Cancel resenting the total amount of counts acquired COM1 during the reading time per well COM3 By checking Calculate RLU/s the total amount Instrument Configuration of counts will be divided by the respective reading time ▼ Dispenser 1 (0µl) installed ----- Get -----Dispenser 2 (0µl) installed Configuration ▼ Dispenser 3 (0µl) installed 23. Click <Get Configuration> the available injectors (with their volume) of the Fluorescence installed instrument will be automatically checked as well ✓ Luminescence installed as Temperature Control and PMT Cooling when ✓ Absorbance installed installed 24. Click <OK> Temperature Control installed ☑ PMT Cooling installed 25. Mikrowin only: Click <OK> to close the Installation | Driver dialogue
- 26. The instrument is now ready to use



5.3 Installing Filters

The instrument comes with an excitation and an emission filter slide, each of capable of holding up to 5 filters.

If the instrument is equipped with absorbance reading technology a 450 nm absorbance filter is included.

If the instrument is equipped with fluorescence reading technology a 485/14 nm excitation filter and a 535/25 nm emission filter are included.

In case additional filter are required they can be ordered individually and can easily be installed both physically and in the software.

5.3.1 Excitation filters

 Select Excitation Filter Slide in the Instrument menu



2. Click <Add>



- 3. Define a (descriptive) Name for the new filter
- 4. Check the **Usage**
- 5. Click **<OK>**





 Highlight the new filter in the Unused Filters list and drag it into an empty position of the filter slide



- 7. Open the big flap at the front
- 8. Click <Eject Slide>
- 9. Remove excitation filter slide from the instrument



emission filters

excitation filters

10. Mount the filter(s) into the position(s) defined in the software

for excitation and absorbance filters with diameters of 12.7 mm ($\frac{1}{2}$ inch), 15 mm or 25 mm (1 inch) can be used

12.7 mm filters need to be mounted with a matching adapter (**ID 57194-005**) and a matching clamp ring (**ID 57195-005**)

15 mm filters need to mounted with a matching adapter (**ID 54666-005**) and a matching clamp ring (**ID 34767-005**) as well



excitation filters mounted



- 11. Insert the slide again until the front of the slide is aligned with the front of the instrument
- 12. Click **<OK>**
- 13. Close the front flap



- 14. Click **Calibrate Slide** when more than filter have been installed or the instrument has been set up for the first time
- 15. Highlight the single filter by right-clicking and click **Calibrate Filter** when only a single new filter has been added
- 16. The instrument will test and finally store the optimized lamp energy for each filter according to its transmission specifications; this may take a few minutes

Do not switch off the instrument during this procedure!

17. Click **<OK>** (once the dialog is black and active again)



5.3.2 Emission filters

1. Select Emission Filter Slide in the Instrument menu

File View	Instrument Tools Help	2		
🗅 😂 🖬 👌	Properties			
Default	Load Plate Unload Plate		🕨 Start Mea	isurement
	Injector Settings Prime Wash	2	3	4
Protocol Des	Refresh	Sample	Sample	Sample
6	Unload Injector Excitation Filter Slide Emission Filter Slide Plate Editor	Sample	Sample	Sample
ReRun & Ec	Boot Instrument	Sample	Sample	Sample









6. Instrument Control and Evaluation Software

6.1 ICE Directories and Files

The directories for data and parameter files are defaulted as described below. Any accessible directory on the computer and the local network can be selected though when saving data and parameter files using the "**Save** ... **File As**..." command.

Default directories

- Data files
 My Documents\ICE\DataTriStar2
 - Protocol files
 My Documents\ICE\ParaTriStar2

In consequence each Windows user has own directories containing his data and protocol files.

File Names

There is no limitation in naming data and protocol files other than the Windows conventions.

Data file names are to be defined prior to measurement start. Renaming is possible using the **"Save Data File As...**" command producing a copy of the data file with a new name.

Protocol file names are to be defined at the end of creating a protocol. Renaming is possible using the "**Save Protocol File As...**" command producing a copy of the protocol file with a new name.

File Types

ICE works with 5 file types indicated by the respective file name extensions.

- Protocol files have the extension .wgp
- Data files have the extension .wgd
- Standard curve files have the extension .wgs (to be used as reference curves)
- Multiple Analyte profiles have the extension .wgm
- Customized prime sequences have the extension .wge



6.2 ICE User Interface

6.2.1 ICE default set up

tocol Design	A	1 Spl 1/1	2	3	4								
tocol Design	A	Spl 1/1	2	3	4	E .	6	7	0	0	10	1 11	10
0			Spl 2/1	Spl 3/1	Spl 4/1	Spl 5/1	Spl 6/1	Spl 7/1	Spl 8/1	Spl 9/1	Spl 10/1	Spl 11/1	Spl 12/1
	В	Spl 13/1	Spl 14/1	Spl 15/1	Spl 16/1	Spl 17/1	Spl 18/1	Spl 19/1	Spl 20/1	Spl 21/1	Spl 22/1	Spl 23/1	Spl 24/1
Run & Edit	с	Spl 25/1	Spl 26/1	Spl 27/1	Spl 28/1	Spl 29/1	Spl 30/1	Spl 31/1	Spl 32/1	Spl 33/1	Spl 34/1	Spl 35/1	Spl 36/1
2	D	Spl 37/1	Spl 38/1	Spl 39/1	Spl 40/1	Spl 41/1	Spl 42/1	Spl 43/1	Spl 44/1	Spl 45/1	Spl 46/1	Spl 47/1	Spl 48/1
ality Check	E	Spl 49/1	Spl 50/1	Spl 51/1	Spl 52/1	Spl 53/1	Spl 54/1	Spl 55/1	Spl 56/1	Spl 57/1	Spl 58/1	Spl 59/1	Spl 60/1
	F	Spl 61/1	Spl 62/1	Spl 63/1	Spl 64/1	Spl 65/1	Spl 66/1	Spl 67/1	Spl 68/1	Spl 69/1	Spl 70/1	Spl 71/1	Spl 72/1
	G	Spl 73/1	Spl 74/1	Spl 75/1	Spl 76/1	Spl 77/1	Spl 78/1	Spl 79/1	Spl 80/1	Spl 81/1	Spl 82/1	Spl 83/1	Spl 84/1

The next figure shows the default start-up screen of ICE.

To return to the default layout after any changes may have been made check **Navigation Panel** and **TriStar²** in the **View** menu.

Instrume	ent Control a	nd Evalua	tion					Tables, o	-	at the second
File Vie	w Instrum Navigatio	nent To n Panel	ools Help							
Defau	CentroLIA TriStar2	pc	ile Nar	ne	🕨 🕨 Star	t Measurem	ent			
	Protocol V	Vizard	s							
E	Data			2	3	4	5	6	7	8
Proto	Result SampleId Standards		πple	Sample	Sample	Sample	Sample	Sample	Sample	Sample
T	Result Mo	de	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
ReRun 8	& Edit	с	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
		D	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
		E	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
				- 5 1	- 1	- 81	-0			12



6.2.2 File menu

The File menu contains commands to open and save data and protocol files.

File	View Instrument	Tools	Help
	New	Ctrl+N	l.
1	Open Protocol File	Ctrl+0	ř.
	Save Protocol File	Ctrl+S	;
	Save Protocol File As		
	Open Data File		
	Save Data File		
	Save Data File As		
	Export		
9	Print	Ctrl+P	0
B,	Print Preview		
	Exit		

New

Open Protocol File...

erases data display to start a new measurement

opens an existing protocol



Save Protocol File	saves loaded protocol file
Save Protocol File As	saves loaded parameter settings with a new name
Open Data File	opens an existing measurement
Save Data File	saves displayed data
Save Data File As	saves displayed data with a new name
Export	exports the data set as EXCEL file according to the settings made in the protocol
Print	prints the selected data set shown on the screen
Print Preview	displays a preview of the print-out
Exit	closes ICE software



6.2.3 View menu

The View menu defines how the user interface and data are displayed.



Navigation Panel	sho
TriStar ²	adj
Protocol Wizard	sta
Data	dis
Result	dis
Sample ID	dis
Standards	dis
Result Mode	tos

shows/hides navigation panel on the left
adjusts user interface for TriStar ²
starts wizard for protocol creation
displays raw data (RLU or RLU/s)
displays calculated data
displays sample IDs
displays standard concentrations
to select the content of the result display





6.2.4 Instrument menu

In the **Instrument** menu basic instrument settings and communication may be accessed.



Properties

instrument driver settings

pmPort	ОК
СОМ1 👤	Cancel
T601002	
COM3	
strument Configuration	
✓ Dispenser 1 (0µl) inst.	alled Gat
🔽 Dispenser 2 (0µl) inst	alled Configuration
Dispenser 3 (0μl) instant	alled
Fluorescence installed	
 Luminescence installer 	d
 Absorbance installed 	
 Temperature Control in 	nstalled
PMT Cooling installed	

Load Plate	moves plate into the instrument
Unload Plate	moves plate out of the instrument
Injector Settings	general setting for wash and prime sequences
Prime	starts the priming sequence (filling the lines)
Wash	starts the washing sequence (cleaning the lines)
Refresh	injects once to fill the tip (e.g. after longer periods of idleness)
Unload Injector	starts the unloading sequence (recovering reagents back into the reservoir)



Excitation Filter Slide Emission Filter Slide Plate Editor dialogue for definition and positioning of excitation filters dialogue for definition and positioning of emission filters dialogue for definition of microplate dimensions

e Editor				
Name				
Copy of Berthold 96			~	Cancel
Berneter				New Plate
Number of rows (1 - 32):	8			Сору
Number of columns (1 ·	48): 12	2		Apply
Height of the plate in (m	m): 14	1.7		Bernove Plate
Stacking height in (mm):	1:	3.2		Tranove Flate
Distance from corner a	and from well to	well in (mm)		
11.5	14.4 14.4 12 12 14.4 12 14.4 12 14.4 14.4	3 1 0]	
Usage Centro	Mithras	🔽 Twin	kle	

Note: only 6 to 384 well plates are supported in the TriStar²

Note: only plate heights of up to 21 mm are supported in the TriStar²

Boot Instrument	establishes communication and boots instrument
Shipping Brace	moves XY table to a position enabling the insertion of the transportation lock



6.2.5 Tools menu

In the **Tools | Options** menu you can define the default root directory for the protocol (*ParaTriStar2*) and data (*DataTriStar2*) folders.



The **Tools | Prime Customize** menu user and/or application specific prime procedures can be defined.

For the setting and options please read chapter "Priming Tubings"



6.2.6 Help menu

The **Help** menu allows you to view basic software information.

■ ICE	×
Instrument Control and Evaluation Version 1.0.4.0 Copyright © Berthold Technologies 2007-2011 Berthold Technologies	
ICE Software for Instrument Control and Evaluation Application Status: Advanced ICE (1.0.4.0) AreaBase (1.0.2.0) Utilities (1.0.3.0) ParaLayer (1.0.3.0) DeviceLayer (1.0.3.0) DataLayer (1.0.3.0) Office2007Renderer (1.0.1.0) TriStar (1.0.0.0)	E
LybraVb (1.3.1.0)	+
<u>O</u> K	



7. Operation with ICE

Running measurements on the TriStar² is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated and Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

7.1 Adding and Editing Microplate Dimensions

Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer's most recent information for exact dimensions of the microplates.

Microplates must be defined in the plate editor prior to defining a measurement protocol.

1. Click Plate Editor in the Instrument menu



2. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**

Name Bethold 96 - No 23300/23302		Cance
		New Pla
Parameters		
Number of rows (1 - 32):	8	Сору
Number of columns (1 - 48):	12	Apply
Height of the plate in (mm):	14.4	
Stacking height in (mm):	135	Remove P
11.24		



- 3. Assign a (descriptive) Name
- 4. Insert the **Number of rows**, e.g. **8** for a 96 well plate
- 5. Insert the Number of columns, e.g. **12** for a 96 well plate
- Insert the total Height of the microplate most 96 and 384 well plates are between 14 and 15.5 mm
- 7. Insert the **Stacking height** of the microplate

the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height

- 8. Insert the distance between the left outer edge of the plate and the center of well A1
- 9. Insert the distance between to upper outer edge of the plate and the center of well A1
- 10. Insert the distance between the well centers of consecutive rows (vertical well distance)
- 11. Insert the distance between the well centers of consecutive columns (horizontal well distance)
- 12. Check the usage TriStar2

you may check additional instruments in case you have multiple instruments in operation

- 13. Click <Apply>
- 14. Click **<OK>**
- 15. The plate can now be used in the protocol files

Name		OK
Copy of Berthold 96 - No:23300/	23302	Cancel
Paramatere		New Pla
Number of rows (1 - 32):	8	Сору
Number of columns (1 - 48):	12	Apply
Height of the plate in (mm):	14.4	Remove P
Stacking height in (mm):	13.5	Temover
Distance from corner and from	well to well in (mm)	
	14.38	
	1/1 2 3	
11.24		
	E Q Q Q	
	9	
- Usage		



7.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

7.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Click icon **Protocol Design** in the left-hand **Nav-igation** bar

the navigation bar will appear in a new design

17. Click the **new** icon in the left-hand **Navigation** bar

again, the navigation bar will appear in a new design

for editing an existing protocol use the edit icon

 The start up screen of the protocol wizard will show up Click <Next>









- 19. Enter a (descriptive) Name for your protocol
- 20. Define the **reading orientation**: by column or by row
- 21. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
- 22. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 23. Check **Temperature** to activate the temperature control for this protocol
- 24. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

25. Click <Next>

Gene	aral Page of Raw Data Protocol sin sert parameters for measurement.	ngle endpoint
	General Protocol name Measuring orientation	single endpoint by row
	Keep Plate after Measurement	Meander
	Temperature Control	
	✓ Temperature	37.0 *Celsius
	Robot Multi Plate Data File Mode	Barcode
		< Back Next > Cancel

26. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 27. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement







- 28. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

29. Click <Next>

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

30. Double-click *Dispense* in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

31. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)



Protocol Wizard
Measurement Page Define Measurement Sequences.
Measurement: Sequence Vert Shake Vert
Dispense Properties
Injector: 1 OK
Volume [µ]: 100 ↓ Cancel Speed: 2 ▼
Operation Mode: 💿 By plate 💿 By well

Perform on first plate repeat only



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32. Double-click *Delay* in case an delay/incubation time is required
Duration 0.1 to 3600 s
Operation Mode by plate or by well

Deration Mode	by plate or by well

- 33. Click **<OK>**
- 34. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well

35. Click **<OK>**

		(0.10-30008)	UK
Operation Mode:	By plate	🔘 By well	Cancel
Perform on first pla	te repeat only		

Delay Properties

Duration:	5	(0.10 - 3600 s)	ОК
Speed: 💿 Slow	Normal	🔘 Fast	Cancel
Diameter:	2	(0.10 - 5 mm)	
Type: 🔘 Linear	Orbital	Ouble orbital	
Operation Mode:	By plate	🔘 By well	
Perform on first plate i	repeat only		

36. Double-click *Endpoint* in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Operation Mode	by plate or by well

27	Click	-OK-
57.		

38. Double-click *Endpoint* in the Fluorescence section for a fluorescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Operation Mode	by plate or by well

39. Click **<OK>**

minescence		1.15	Transfer of	X
Name:	luminescence 1s	I		ОК
Counting Time:	1	(0.05 - 600 s)		Cancel
Emission Filter:	No Filter - Slot A	5	•	
Operation Mode:	By plate	By well		
Second Measure	ment			
Emission Filter;	No Filter - Slot A	5		

Name:	Fluorescence485/535	OK
Counting Time:	0.10 (0.05 - 600 s)	Cancel
Lamp Energy:	35	
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2 -	
Emission Filter:	F535 (FITC Fluorescein) - Slot A2	
Second Measurem	ient	
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2 +	
Emission Filter.	F535 (FITC Fluorescein) - Slot A2 *	



40. Double-click *Endpoint* in the Absorbance section for an absorbance reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or Auto

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter select from the list

Note: filters must be defined prior in the Instrument menu

Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

- 41. Click **<OK>**
- 42. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

43. Click <Next>







44. Define Export settings

Type your Header specific for this protocol

Select the data set by dragging from left to rightSample IDsample informationMeasurement DatareadingsResultcalculated dataErrorany error codesOverlaywell informationStatisticsmeasurement settings

Type your Footer specific for this protocol

Define Directory for the export file

Check if Automatic Export is required

- 45. Click <Next>
- 46. Define Print settings

Select the data set by dragging from left to rightPage Headerfile namesMeasurement DatareadingsStatisticsmeasurement settingsResultscalculated dataOverlaywell informationAll Curveskinetics curvesZoomed Curveszoomed view of curves

Define page orientation and margins

Check if Automatic Print-out is required

- 47. Click <Next>
- 48. Click <Finish>





xport Page Define sett	ings for data export.		10
Header:	my header		0
Content	 → Aualtable Items → Result - Sampleid - Error - Overlay) = Selected Rons → Meaurement Data → Statistics	
Footer:	my foster		•
Directory:	C-\Users\\Deaktop		_
[Automat	to Export after Measurement		
		< Back	Next

Define settings for	printout.					13-
© Avalded Inters — Reads — Statistical — Statistical — A Conves Zoomet Curves					elected items — Pago Header — Massurement Data — Statistics	
Orientation	Margin	s (mm)	Dialat	100		
 Landscape 	Top:	15.00	Bottom:	15.00		
	tter Measure	ment				

- 49. Define the protocol file name
- 50. Click **<Save>**

Organize 🔻 New folder		框•
Favorites Desistop Downloads Standards Lohanies Uchranies Occuments Muic Pictures Videos	Documents library PariToSan2 Name Default.wgp	Arrange by: Folder – Date modified 03/12/2012 14:11
File name: Eingle endpoint wop Save as type: protocol files (*.wgp)	• • • [



7.2.2 Measurement with a Single Endpoint protocol

The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 8</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used

2. Enter a **file name** under which the measurement is to be stored

File View Instrum	ment T	ools Help			
) 🞽 🖬 🖪 🕘					
singleLumiendpoint	▼ E	nter File Nam	ie	Start Mea	isurement
Default					
ingleLumiendpoint	9	Statistics			
1		1	2	3	4
Protocol Design	A	Sample	Sample	Sample	Sample
-		Granda	Consula	Comple	Comple

Instrument Contr	ol and Eva	luation	
File View Ins	trument	Tools Help	
0 🗃 🖬 🥔 (0		
singleLumiendpoi	nt 🕨	readABC	Start Measurement
	Data	Statistics	Start Measurement

- 3. Click <Start Measurement>
- 4. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

5. Click **<OK>**





 The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

gleLumiendpoin		• 10	284ba		Stop Me	surement								
	Det		abilita											
1	E	-	1	2	3	4	5	6	7	1	9	10	11	12
Accol Design		A	168	158	158	168	135	168	Serple	Sergie	Sample	Sergie	Sample	Sample
B.		1	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
dRun & Edt		с	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Songle	Sample	Sanple
R		D	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Sample	Sample	Sanple
uality Check		ε	Sample	Sample	Sangle	Sample	Sample	Sarple	Sample	Sanple	Sample	Sargio	Sample	Sanple
	,	F	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
		G	Sample	Sample	Sample	Sample	Sample	Sargia	Sample	Sample	Sample	Sample	Sample	Sample
Marrie		н	Sample	Serple	Serple	Sergie	Sample	Samples	Sergie	Sergie	Sample	Sangle	Sarple	Sercie

7. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

File View	Instrument	Tools Help			
🗅 💕 🖬 🍦	Propert	ies			
Default	Load Pl Unload	late Plate		🕨 Start Mea	isurement
	Injector Prime Wash	r Settings	2	3	4
Protocol Des	Refresh Unload	Injector	Sample	Sample	Sample
B	Excitati Emissic Plate Ec	on Filter Slide on Filter Slide ditor	Sample	Sample	Sampl
ReRun & Ec	Boot In	strument	Sample	Sample	Sample



7.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

7.3.1 Defining a Dual Label protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Nav-igation** bar

the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

again, the navigation bar will appear in a new design

for editing an existing protocol use the edit icon

 The start up screen of the protocol wizard will show up Click <Next>









- 4. Enter a (descriptive) Name for your protocol
- 5. Define the **reading orientation**: by column or by row
- 6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
- 7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 8. Check **Temperature** to activate the temperature control for this protocol
- Define the target temperature the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 10. Click <Next>
- 11. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 12. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement









- 13. Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

14. Click <Next>

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

15. Double-click **Dispense** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)







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X

OK

Cancel

17. Double-click *Delay* in case an delay/incubation time is requiredDuration0.1 to 3600 s

Operation Mode	by plate or by well

- 18. Click **<OK>**
- 19. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well
20. Click <ok></ok>	

Duration:	3	(0.10 - 3600 s)	ок
Operation Mode:	By plate	🔿 Bywell	Cancel
Perform on first pl	ate repeat only		

Delay Properties

Luminescence

Counting Time:

Emission Filter:

Operation Mode

Second Measurement

Name

Firefly

10.00

No Filter - Slot A5

No Filter - Slot A5

By plate

nake Properties		- Anno	×
Duration:	5	(0.10 - 3600 s)	ОК
Speed: Slow	Normal	🔿 Fast	Cancel
Diameter:	2	(0.10 - 5 mm)	
Type: 🔘 Linear	Orbital	Oouble orbital	
Operation Mode:	By plate	🔘 By well	
Perform on first plate	repeat only		

(0.05 - 600 s)

By well

•

21. Double-click *Endpoint*, e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Operation Mode	by plate or by well

- 22. Click **<OK>**
- 23. Once more double-click *Endpoint*, e.g. in the Luminescence section for a luminescence reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Operation Mode	by plate or by well

X Luminescence Renilla Name ОК Counting Time: 10.00 (0.05 - 600 s) Cancel Emission Filter: No Filter - Slot A5 -Operation Mode By plate By well Second Measurem Emission Filter: No Filter - Slot A5

24. Click **<OK>**



25. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click <Next>



- 27. Select the calculation to perform with the 2 measurements
- 28. Click <Next>

elect type of calculation of Multilat	bel Measurements.	
	Type of calculation Read 1 / Read 2 Read 2 / Read 1 Read 1 - Read 2	O
	 Read 1 / Read 2 Read 2 / Read 1 Read 1 - Read 2 Read 2 - Read 1 	

29. Define Export settings

Type your **Header** specific for this protocol

Select the data set by dragging from left to rightSample IDsample informationMeasurement DatareadingsResultcalculated dataErrorany error codesOverlaywell informationStatisticsmeasurement settings

Type your Footer specific for this protocol

Define Directory for the export file

Check if Automatic Export is required

Header:	my header		0
			-
Content:	 → Available tems → Renut → Sampleid → Error → Overlay 	Statuted tens - Statetice - Statetice	
Footer:	my footer		0
Drectory:	C.\Users\\Desktop		
V Automat	tic Export after Measurement		



- 30. Click <Next>
- 31. Define Print settings

y dragging from left to right
file names
readings
measurement settings
calculated data
well information
kinetics curves
zoomed view of curves

Define page orientation and margins

Check if Automatic Print-out is required

- 32. Click <Next>
- 33. Click <Finish>



Protocol Wizard	the second se	
Π	Now you have inserted all necessary parameters to finish creation of Raw Data Protocol .	
	R	
Ц	1-91	
	Press Finish to save protocol.	r Back Frysh Corost

- 34. Define the protocol file name
- 35. Click <Save>

Organize 🔻 New folder			i≣ •
Favorites Desktop	î	Documents library ParaTriStar2	Arrange by: Folder
Sownloads	-	Name	Date modified
3 Recent Places		Default.wgp	03/12/2012 14:1
Libraries			
Documents			
Music			
Pictures			
Videos			
		* <u></u>	
File name: single endpoint.wop			
Save as type: protocol files (*.wop)			



7.3.2 Measurement with a Dual Label Assay protocol

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 8</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the protocol to be used

2. Enter a **file name** under which the measurement is to be stored

Instrument Control a	and Evalua	tion			
File View Instrum	ment To	ools Help			
🗋 🞽 🖬 🥔 🔘					
singleLumiendpoint	▼ Ei	nter File Nam	e	Start Mea	isurement
Default					
singleLumiendpoint	S	tatistics			
1		1	2	3	4
Protocol Design	A	Sample	Sample	Sample	Sample
0	в	Sample	Sample	Sample	Sample



- 3. Click <Start Measurement>
- 4. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

5. Click **<OK>**





6. The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

You may switch between the two readings by clicking on the arrows

	F	irefly	Þ
3	4	5	6
1465	Sam	ple Sample	e Samp

7.	Select Unload Plate in the Instrument menu to
	retrieve the microplate (still in measurement posi-
	tion) and remove it from the instrument

label		• 1	ustend		Stop Me	surement								
	Det	. 5	labelca											
	13	6.6	1	of1 b b		4 Firefly		>						
			1	2	3	4	5	6	7		9	10	11	12
new		٨	1742	1624	1405	Sample	Sanple	Sangle	Sample	Sanple	Sample	Sanple	Sarple	Sanple
Z			Sample	Sample	Sample	Sanple	Sample	Sample	Sample	Sanple	Sample	Sangle	Sample	Sample
edit		с	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Sample	Sample	Sanple
9		D	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Sanple	Sample	Sample
DECK		ε	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Sample	Sançle	Sample
	,	F	Sample	Sample	Sample	Sanple	Sample	Sample	Sample	Sanple	Sample	Sanple	Sample	Sample
		G	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sample	Sample	Sample	Sample
		н	Sample	Sample	Sample	Sample	Sample	Sample	Sanple	Sergie	Sample	Sonple	Sample	Sample

File View	Instrument	Tools Help			
🗅 😂 😡 d	Propert	ies	-		
Default	ault Load Plate Unload Plate			🕨 Start Mea	isurement
	Injector Prime Wash	r Settings	2	3	4
Protocol Des	Refresh	Injector	Sample	Sample	Sample
6	Excitatio Emissio Plate Ec	on Filter Slide on Filter Slide ditor	Sample	Sample	Sample
ReRun & Ed	Boot In	strument	Sample	Sample	Sample



7.4.1 Defining a protocol for a kinetic measurement

seconds up to minutes, e.g. enzyme kinetics and Calcium influx

If you want to use an already existing protocol you may proceed with the next paragraph.

A kinetic measurement mode is appropriate for fast kinetics assays lasting over several

1. Click icon **Protocol Design** in the left-hand **Nav-igation** bar

the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

again, the navigation bar will appear in a new design

for editing an existing protocol use the edit icon

 The start up screen of the protocol wizard will show up Click <Next>

7.4 Kinetic Measurement

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- 4. Enter a (descriptive) Name for your protocol
- 5. Define the **reading orientation**: by column or by row
- 6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
- 7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 8. Check **Temperature** to activate the temperature control for this protocol
- Define the target temperature the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 10. Click <Next>
- 11. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 12. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement









- Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

14. Click <Next>

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

15. Double-click **Dispense** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)






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(0.10 - 3600 s) OK

O By well

Ouble orbital

O By well

Cancel

17. Double-click *Delay* in case an delay/incubation time is requiredDuration 0.1 to 3600 s

Operation Mode	by plate or by well

- 18. Click **<OK>**
- 19. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well
20. Click <ok></ok>	

Perform on first pla	te repeat only		
Shake Properties		-	x
Duration:	5	(0.10 - 3600 s)	ОК
Speed: Slow Diameter:	Normal	Fast (0.10 - 5 mm)	Lancel

Delay Properties

Operation Mode

C Linear

Perform on first plate repeat only

Type:

Operation Mode

3

By plate

Orbital

By plate

Duration

21. Double-click *Kinetic* in the Luminescence section

for a	luminescence	kinetic	reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake ins	stead of Delay if needed

Delay	0 to 600 sec
Repeats	(are calculated)
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

22. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

Name:	LumiKinetic		OK
Total Time:	10.00	(1 - 604800 s)	Cancel
Counting Time:	1.00	(0.05 - 600 s)	
🔲 Use Shake inste	ad of Delay		
Delay:	0.00	(0 - 600 s)	
Repeats:	11	(1 - 50000)	
Emission Filter:	No Filter - Slot	A5 🔹	
Second Measure	ement		
Emission Filter.	No Filter - Slo	t A5 👻	



23. Double-click *Kinetic* in the Fluorescence section for a fluorescence kinetic reading

	-
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake ins	stead of Delay if needed
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
Note: filters must be	defined prior in the Instru-

Note: filters must be defined prior in the Instrument menu

Operation Mode by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

24. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

25. Double-click *Kinetic* in the Absorbance section for an absorbance kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)

Counting Time 0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay 0 to 600 s

Repeats (are calculated)

Lamp Energy 0 to 100 % or *Auto*

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Note: filters must be defined prior in the Instrument menu

26. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way



orescence Kinetics		Contraction of the second	-×
Name:	FluoKinetic		ОК
Total Time:	5	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Use Shake instea	ad of Delay		
Delay:	0.00	(0 - 600 s)	
Repeats:	9	(1 - 50000)	
Lamp Energy:	100	an an an an an an an an	-Q
Lamp Energy: Excitation Filter:	100 F485 (FITC Fi	uorescein) - Slot A2	-0
Lamp Energy: Excitation Filter: Emission Filter;	100 F485 (FITC Fil F535 (FITC Fil	uorescein) - Slot A2	Ģ
Lamp Energy: Excitation Filter: Emission Filter:	100 F485 (FITC FI F535 (FITC Fi ment	uorescein) - Slot A2	Ų
Lamp Energy: Excitation Filter: Emission Filter: I Second Measure Excitation Filter:	100 F485 (FITC Fi F535 (FITC Fi ment F485 (FITC Fi	uorescein) - Slot A2	Ų

Name:	Kinetic		ОК
Total Time:	20	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
🔽 Use Shake instead	l of Delay		
Delay:	1	(0 - 600 s)	
Repeats:	19	(1 - 50000)	
Lamp Energy:	Auto) <u>, , , , , , , , , , , , , , , 0</u>	V Auto
Measurement Filter:	F450 (Absor	bance) - Slot A1 🔹	

27. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

28. Click <Next>



29. Define Export settings

Type your Header specific for this protocol

Select the data set by dragging from left to rightSample IDsample informationMeasurement DatareadingsResultcalculated dataErrorany error codesOverlaywell informationStatisticsmeasurement settings

Type your **Footer** specific for this protocol

Define Directory for the export file

Check if Automatic Export is required

30. Click <Next>

Define set	ings for data export.		13.
Header:	my header		0
Content	Available items Penut Sançleid Error Overlay	Salecter tens	
Footer:	my footer		•
Directory:	C.\Users\\Desktop		
[Astoma	tic Export after Measurement		



31. Define Print settings

Select the data set by	y dragging from left to right
Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

Define page orientation and margins

Check if Automatic Print-out is required

- 32. Click <Next>
- 33. Click <Finish>





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1

35. Click **<Save>**

Organize 🔻 New folder			⊯ •
	E	Documents library ParaTriStar2 Name Default.wgp	Arrange by: Folder + Date modified 01/12/2012 14:13
File name:	-	• [
Ship at base anotarel file (* une)			



7.4.2 Kinetic measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 8</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

8. Select the **protocol** to be used

9. Enter a **file name** under which the measurement is to be stored

File View Instrur	ment To	ools Help			
) 🞽 🖬 🎒 🎯					
singleLumiendpoint	▼ Ei	nter File Nam	e	Start Mea	isurement
Default					
ingleLumiendpoint	SI	atistics			
1		1	2	3	4
Protocol Design	A	Sample	Sample	Sample	Sample

Instrument Cont	trol and Eva	luation		
File View In	strument	Tools	Help	
0 🞽 🖬 ∂	0			
singleLumiendpo	int 🔤	readAB	c	Start Measurement
	Data	Statistic	s	Start Measurement

- 10. Click **<Start Measurement>**
- 11. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

12. Click **<OK>**





 The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

You may switch between the individual readings by clicking on the arrows

Instrument Control	and Evaluat	ion					
<u>File View</u> Instru	iment <u>I</u> o	ols <u>H</u> elp					
LumiKineticw2xInj	+ lu	mikinetics		Stop Mea	surement		
	Data Sta	atistics Kine	tics				
	1 IA A	8	of 32 🕨 🔰	7.000			>
		1	2	3	4	5	6
new	► A	188	168	Sample	Sample	Sample	Sample
Z	в	Sample	Sample	Sample	Sample	Sample	Sample
edit	c	Sample	Sample	Sample	Sample	Sample	Sample
	D	Sample	Sample	Sample	Sample	Sample	Sample

- 14. You also choose to view the curves by clicking the *Kinetics* tab
- 15. The scale of the axes can be changed by rightclicking into the curves and selecting **Options...**

Y Axis Minimum:	Auto	© Fix	0
Y Axis Maximum:	Auto	© Fix	416
X Axis Minimum:	Auto	© Fix	0
X Axis Maximum:	Auto) Fix	30

Instrument Contro	ol and Evaluat	ion					
<u>File View</u> Inst	rument <u>I</u> o	ols <u>H</u> elp					
)						
LumiKineticw2xInj	+ lur	nikinetics		Stop Measur	rement		
	Data Sta	stistics Kinetics]				
		1	2	3	4	5	6
new	► A	mm		un			
17	В						
edit	с						
\bigcirc	D						
back						I	

16. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

	4	5	6 7
~~	www	·	
_			Zoomed Curves All Curves
		\checkmark	LB1
			Options

S . S .	ur Toor Tech		
iKinetica/2dq	Turvikientics Step Mei	surement	
D	ata Statistica Kinatica		
new	400		
	360 -		
/	340		
	320		
	280		
	260		
	220 A.		
back	200	$\wedge \wedge \rightarrow \rightarrow$	and made
	160		- V V~
	140		
	100		
	80		
	40 -		
	20		
	0 1 2 3 4 5 6 1	8 9 10 11 12 13 14 15 16 17 18 19	20 27 22 23 24 25 26 27 28 29 30

To un-zoom right-click into the zoomed view and select All Curves





17. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

File View	Instrument Tools	Help		
0 😂 🖬 d	Properties			
Default	Default Load Plate Unload Plate		🕨 Start Mea	isurement
	Injector Settings Prime Wash	2	3	4
Protocol Des	Refresh Unload Injector	Sample	Sample	Sample
Excitation Filter Slide Emission Filter Slide Plate Editor		de Sample le	Sample	Sample
ReRun & Ec	Boot Instrument	Sample	Sample	Sample



7.5 Repeated Measurement

A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

7.5.1 Defining a protocol for a repeated measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Nav-igation** bar

the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

again, the navigation bar will appear in a new design

for editing an existing protocol use the edit icon

 The start up screen of the protocol wizard will show up Click <Next>









- 4. Enter a (descriptive) Name for your protocol
- 5. Define the **reading orientation**: by column or by row
- 6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
- 7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 8. Check **Temperature** to activate the temperature control for this protocol
- Define the target temperature the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 10. Click <Next>
- 11. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 12. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement









- Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

14. Click <Next>

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check
 mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

15. Double-click **Dispense** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)







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Operating Manual

17. Double-click *Delay* in case an delay/incubation time is requiredDuration0.1 to 3600 s

Operation Mode	by plate or by well

- 18. Click **<OK>**
- 19. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well
20. Click <ok></ok>	

Duration:	3	(0.10 - 3600 s)	ок
Operation Mode:	By plate	O By well	ancel
Perform on first pla	te repeat only		

Duration:	5	(0.10 - 3600 s)	OK
Speed: Slow	Normal	🔘 Fast	Cancel
Diameter:	2	(0.10 - 5 mm)	
Type: 🔘 Linear	Orbital	Double orbital	
Operation Mode:	By plate	🔘 By well	
Perform on first plate i	repeat only		

21. Double-click *Repeated* in the Luminescence section for a luminescence repeated reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Injector 1, ...2, ...3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	0 means prior to a measurement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

22. Click <OK>

a second repeated operation may be added, e.g. for ratiometric applications (BRET)

00.00 0 4.14 Filter - Slot A	(1 - 604800 s) (0.05 - 600 s) (124.14 - 6000 (1 - 50000) 5	s)	Cancel
00.00 0 4.14 Filter - Slot A	(1 - 604800 s) (0.05 - 600 s) (124.14 - 6000 (1 - 50000) 5	5)	Cancel
0 4.14 Filter - Slot A!	(0.05 - 600 s) (124.14 - 6000 (1 - 50000) 5	s)	
4.14 Filter - Slot A!	(124.14 - 6000 (1 - 50000) 5	s)	
Filter - Slot A	(1 - 50000) 5		
Filter - Slot A	5		
		-	
)	(0 - 29)		
	1	(0 - 29) • •	(0 - 29)) *



23. Double-click *Repeated* in the Fluorescence section for a fluorescence repeated reading

	1 5
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
<i>Note:</i> filters must be ment menu	defined prior in the Instru-

Injector 1, ...2, ...3

Check Use Injector for an injection within the repeated cycle

Injector Cycle	0 means prior to a meas- urement
Volume	10 to 100 μL
Speed	1 to 5
Operation Mode	by plate or by well

lame:	Huorepeated		UK
Total Time:	300.00	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Cycle Time:	33.33	(33.33 - 6000 s)	
Repeats:	10	(1 - 50000)	
Lamp Energy:	100		Q
Excitation Filter:	F485 (FITC Flu	Jorescein) - Slot A2 🔹	
Excitation Filter: Emission Filter:	F485 (FITC Fil	uorescein) - Slot A2 🔹 🔻	
Excitation Filter: Emission Filter:	F485 (FITC Fil F535 (FITC Fil 535 (FITC Fil 1)	uorescein) - Slot A2 🔹 👻	
Excitation Filter: Emission Filter: Injector 1 Injector 2 Use Injector Injector Cycle:	[F485 (FITC Fl [F536 (FITC Fl 2] Injector 3]	uorescein) - Slot A2	
Excitation Filter: Emission Filter: Injector 1 Injector 2 Use Injector Injector Cycle: Volume:	[F485 (FITC Fli [F535 (FITC Fli 2] Injector 3] 0 100	uorescein) - Slot A2	

24. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (FRET)



25. Double-click *Repeated* in the Absorbance section for a absorbance repeated reading

•	•
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 % or <i>Auto</i>

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Check Reference Measurement if needed

Reference Filter select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, ...2, ...3

Check Use Injector for an injection within the re-

peated eyele
0 means prior to a meas- urement
10 to 100 µL
1 to 5
by plate or by well

26. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications

Name:	AbsRepeated	d	ОК
Total Time:	600	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Cycle Time:	60	(35.29 - 6000 s)	
Repeats:	11	(1 - 50000)	
Lamp Energy:	Auto	<u>, , , , , , , , , , , , , , , , , , , </u>	🔽 Auto
Measurement Filter:	F450 (Absorb	ance) - Slot A1 🔹	
Reference Measure Reference Filter:	ement F450 (Absorb	ance) - Slot A1 *	
Reference Measure Reference Filter:	ement [F450 (Absorb	ance) - Slot A1 +	
Reference Measure Reference Filter: Injector 1 Injector 2	F450 (Absorb	ance) - Slot A1 🔹 👻	
Reference Measure Reference Filter: Injector 1 Injector 2 Use Injector	F450 (Absorb	ance) - Slot A1 👻	
Reference Measure Reference Filter: Injector 1 Injector 2 Use Injector Injector Cycle:	F450 (Absorb	ance) - Slot A1 *	
Reference Measure Reference Filter: Injector 1 Injector 2 Use Injector Injector Cycle: Volume:	F450 (Absorb	ance) - Slot A1 *	
Reference Measure Reference Filter: Injector 1 Injector 2 Use Injector Injector Cycle: Volume: Speed:	F450 (Absorb Injector 3 0 100 2	ance) - Slot A1 *	



27. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

28. Click <Next>



29. Define Export settings

Type your Header specific for this protocol

Select the data set by dragging from left to rightSample IDsample informationMeasurement DatareadingsResultcalculated dataErrorany error codesOverlaywell informationStatisticsmeasurement settings

Type your **Footer** specific for this protocol

Define Directory for the export file

Check if Automatic Export is required

30. Click <Next>

Export Page Define sett	ings for data export.		-
Header:	my header		O
Content	Available tems Result Sancleid Eror Overfay	Second tens Meaurement Data Saturca	
Footer:	my footer		0
Directory:	C.\Users\\Desktop		
💟 Automat	ic Export after Measurement		



31. Define Print settings

Select the data set by	y dragging from left to right
Page Header	file names
Measurement Data	readings
Statistics	measurement settings
Results	calculated data
Overlay	well information
All Curves	kinetics curves
Zoomed Curves	zoomed view of curves

Define page orientation and margins

Check if Automatic Print-out is required

- 32. Click <Next>
- 33. Click <Finish>





- 34. Define the protocol $\ensuremath{\textit{file}}\xspace$ name
- 35. Click **<Save>**

Organize 🔻 New folder			⊯ •
	E	Documents library ParaTriStar2 Name Default.wgp	Arrange by: Folder + Date modified 01/12/2012 14:13
File name:	-	• [
Ship at base anotarel file (* une)			



7.5.2 Repeated measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 8</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used

2. Enter a **file name** under which the measurement is to be stored

Instrument Control	and Evalua	tion			
File View Instru	ment To	ools Help			
) 🗳 🖬 🖨 💿					
singleLumiendpoint	▼ Ei	nter File Nam	e	Start Mea	isurement
Default	-				
singleLumiendpoint	S	tatistics			
1		1	2	3	4
Protocol Design	A	Sample	Sample	Sample	Sample
0	В	Sample	Sample	Sample	Sample

Instrument Con	trol and Eva	luation	
File View In	strument	Tools Help	
0 🐸 🛛 ∂	0		
singleLumiendpo	int	readABC	Start Measurement
	Data	Statistics	Start Measurement

- 3. Click <Start Measurement>
- 4. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

5. Click **<OK>**





 The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

You may switch between the individual readings by clicking on the arrows

luaRepeated		fluodualrep5		Stop Mea	surement								
_	Dete	Statutica Rep	baba										
	15	1	2	3	4	5	6	7	1	9	10	11	12
DEW	• •	3318377	3321145	3287559	3274860	3255042	3273880	3225864	3178585	3122221	3080043	3020626	311757
1		3277993	3252989	3258489	3271520	3289495	3266320	3220724	3167317	3125107	3086211	3043681	3101935
edit		3282531	3296254	3292172	3260543	3276936	3252465	3211913	3186881	3190851	3115362	3086058	3077692
9		3245800	3297983	3257365	3240141	3280850	3224722	3229956	3162378	3136714	3116497	3103536	3076716
Dack	e	3250399	3292325	3269243	3261734	3296534	3210738	3260026	3094226	3113889	3145492	3105465	3106017
	F	3272201	3309244	3259099	1238382	3278996	3263344	3240738	3085299	3087204	3174292		
	G												
	,												

- You also choose to view the curves by clicking the *Repeated* tab
- 8. The scale of the axes can be changed by rightclicking into the curves and selecting **Options...**

Y Axis Minimum:	Auto	© Fix	0
Y Axis Maximum:	Auto	⑦ Fix	416
X Axis Minimum:	Auto	© Fix	0
X Axis Maximum:	 Auto 	© Fix	30

duaRepeated	• fluc	sdualrep5		Stop Measur	romont								
	Data Sta	Antica Repeate	2	3	4	5	6	7	1	3	10	11	12
new	• 4												
7													
edit	c												
	D												
back													
	F												
	G												
	н												

9. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Curves**

	4	5	6	7
v	m	· -		_
			Zoomed Curves All Curves	Ī
			LB 1	1
	1		Options	

Vev Instrum	ent Tools Help										
odua≋epested	• Huoduairep5	6	Stop Measur	oment							
	Deta Statutica Repeate	đ									
DEW	anna 1										
	600000										
1	5500000										
-	5000000										
	4500000										
	4000000										
	3500000										
back	3000000										
	2500000 -										
	2000000 -										
	1500000				Š.						°
	1000000 -										
	500000 -										
	0	100	200	300	400	500	600	700	800	900	1000
tocol Design											
e RLU	No Temp.	-	_	_	flui	dualrep5.wgd	1	_	FluodualR	epested.wgp	

To un-zoom right-click into the zoomed view and select All Curves





10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

File View	Instrument Tools Help			
🗅 😂 🖬 👍	Properties	-		
Default	Load Plate Unload Plate		🕨 Start Mea	isurement
	Injector Settings Prime Wash	2	3	4
Protocol Des	Refresh	Sample	Sample	Sample
G	Excitation Filter Slide Emission Filter Slide Plate Editor	Sample	Sample	Sample
ReRun & Ec	Boot Instrument	Sample	Sample	Sample



7.6 Scanning Measurement

A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

7.6.1 Defining a protocol for a scanning measurement

If you want to use an already existing protocol you may proceed with the next paragraph.

1. Click icon **Protocol Design** in the left-hand **Nav-igation** bar

the navigation bar will appear in a new design

2. Click the **new** icon in the left-hand **Navigation** bar

again, the navigation bar will appear in a new design

for editing an existing protocol use the edit icon

 The start up screen of the protocol wizard will show up Click <Next>

BERTHOLD







- 4. Enter a (descriptive) Name for your protocol
- 5. Define the **reading orientation**: by column or by row
- 6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top
- 7. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 8. Check **Temperature** to activate the temperature control for this protocol
- 9. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 10. Click <Next>
- 11. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 12. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement









- Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

14. Click <Next>

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

15. Double-click **Dispense** in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

16. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)







LB 942 TriStar²

Operating Manual

17. Double-click *Delay* in case an delay/incubation time is requiredDuration0.1 to 3600 s

Operation Mode	by plate or by well
au	

- 18. Click **<OK>**
- 19. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well
20. Click <ok></ok>	

Duration:	3	(0.10 - 3600 s)	ОК
Operation Mode:	By plate	By well	ancei
Perform on first pl	ate repeat only		

and tropenses		- Annota	
Duration:	5	(0.10 - 3600 s)	ОК
Speed: Slow	Normal	🔘 Fast	Cancel
Diameter:	2	(0.10 - 5 mm)	
Type: 🔘 Linear	Orbital	Ouble orbital	
Operation Mode:	By plate	🔘 By well	
Perform on first plate r	epeat only		
Dperation Mode:	By plate repeat only	🔘 By well	

21. Double-click *Scanning* in the Fluorescence section for a fluorescence scanning reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Steps	1 to 100 scanning points in one di- rection, the other direction will have the same amount of points

Point Displacement distance between points Select rectangular or round matrix

22. Click **<OK>**

luorescence Scanning	-		×
Name:	ScanningFluo		ОК
Counting Time:	0.10	(0.05 - 600 s)	Cancel
Lamp Energy:	10		
Excitation Filter:	F485 (FITC Fluore	escein) - Slot A2 🔹	
Emission Filter:	F535 (FITC Fluore	escein) - Slot A2 🔹	
Steps:	3	(1 - 100)	
Point Displacement:	2.5	(0.09 - <mark>5</mark> 0 mm)	
Scanning Mode:	Rectangular	Round	



23. Double-click *Scanning* in the Absorbance section for a absorbance scanning reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or <i>Auto</i>

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Note: filters must be defined prior in the Instrument menu

Steps 1 to 100 scanning points in one direction, the other direction will have the same amount of points

Point Displacement distance between points

Select rectangular or round matrix

- 24. Click **<OK>**
- 25. The **sequence of selected operations** will be displayed on the right-hand side

Operations can be moved up or down by clicking on the operation and dragging them to the respective position

Operations can be deleted by highlighting and hitting the DEL key or by dragging to the left

26. Click <Next>







27. Define Export settings

Type your Header specific for this protocol

Select the data set by dragging from left to rightSample IDsample informationMeasurement DatareadingsResultcalculated dataErrorany error codesOverlaywell informationStatisticsmeasurement settings

Type your Footer specific for this protocol

Define Directory for the export file

Check if Automatic Export is required

- 28. Click <Next>
- 29. Define Print settings

Select the data set by dragging from left to rightPage Headerfile namesMeasurement DatareadingsStatisticsmeasurement settingsResultscalculated dataOverlaywell informationAll Curveskinetics curvesZoomed Curveszoomed view of curves

Define page orientation and margins

Check if Automatic Print-out is required

- 30. Click <Next>
- 31. Click <Finish>





Protocol Wi	zard		
xport Page Define sett	ings for data export.		19.
Header:	my header		0
Content:	Haulable tems Pesut Sanyleid Error Overlay	Selected Ions Heaurement Data Statence	
Footer:	ny footer		0
Directory:	C.\Users\\Desktop		
💟 Automat	ic Export after Measurement		
		< Book	Nest > Cancel

Define settings fo	r printout.					2
B Anable tans Pitch Standard Standard A Davie Zoomed Curves					Secold tens - Pape Hade - Manners Data - Secold	
Orientation Portrait	Margine Left:	r (mm) 15.00	Right:	15.00		
Orientation Portrat Landscape	Margine Left: Top:	s (mm) 15.00 15.00	Right: Bottom:	15.00 15.00		

- 32. Define the protocol file name
- 33. Click <Save>

Organize 🔻 New folder		框•
Favorites Desistop Downloads Standards Lohanies Uchranies Occuments Muic Pictures Videos	Documents library PariToSan2 Name Default.wgp	Arrange by: Folder - Date modified 03/12/2012 14:11
File name: Eingle endpoint wop Save as type: protocol files (*.wgp)	• • • [



7.6.2 Scanning measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 8</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

1. Select the **protocol** to be used

2. Enter a **file name** under which the measurement is to be stored

Instrument Control a	nd Evalua	tion			
File View Instrum	ment To	ools Help			
🗋 🞽 🖬 🖨 🔘					
singleLumiendpoint	▪ Er	nter File Nam	e	Start Mea	isurement
Default					
singleLumiendpoint	SI	tatistics			
4		1	2	3	4
Protocol Design	A	Sample	Sample	Sample	Sample
0	в	Sample	Sample	Sample	Sample

Instrument Con	trol and Eva	luation	
File View In	strument	Tools Help	
0 🐸 🛛 ∂	0		
singleLumiendpo	int	readABC	Start Measurement
	Data	Statistics	Start Measurement

- 3. Click <Start Measurement>
- 4. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

5. Click **<OK>**





 The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

You may switch between the individual readings by clicking on the arrows



7. You also choose to view a graphical display by clicking the *Scanning* tab

nningfluo		can2		Stop Measur	omont								
	Data S	teletica Scame	2	3	4	5	6	7	8	9	10	11	12
Den .	• •	82		<u> </u>									
1000	c												
	D												
back													
	F.												
	G												
	н												

8. To get a zoomed view click into the respective wells to highlight them, then right-click and select **Zoomed Areas**

	Zoomed Area
1	All Areas
1	LB1
	Options

Association Control	a and Evaluation		CONTRACTOR OF STREET,		
le Vew Dub	runnent Iocis Help				
anningfluo	+ scan2	Stop Measurement			
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17			and the second data		
12		Contraction of the local division of the loc	AND STREET, ST		4307993 4002438 3877104
		-			3661709 3446314
					3015525 2800130
back					23694736 2369341 2153946
					1930552 1723157 1507763
			and and a		1292368 1076973 861679
				4	545184 430789
					215305
stocol Design					
NE RLU	No Temp.	A05 = 1951437	scanZ.wgd	scenningfluouegp	

To un-zoom right-click into the zoomed view and select All Curves





9. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument

File View	Instrument Tools Help			
0 📽 🖬 👌	Properties	-		
Default	Load Plate Unload Plate		🕨 Start Mea	isurement
	Injector Settings Prime Wash	2	3	4
Protocol Des	Refresh	Sample	Sample	Sample
6	Excitation Filter Slide Emission Filter Slide Plate Editor	Sample	Sample	Sample
ReRun & Ec	Boot Instrument	Sample	Sample	Sample



7.7 Data export and print-out

7.7.1 Direct data export

1. Click on Save Data File As... in the File menu

- 2. Select the file format, e.g. Excel files (*.xls)
- 3. Define the file name without extension
- 4. Select the appropriate folder
- 5. Click <Save>
- 6. Open the *.xls file





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2	Α	22195	11923	20347	19612	26973	31646	23681	
3	В	85	15977	20362	15484	19795	10682	26103	
4	С	11324	30983	24182	26153	5312	11992	18984	
5	D	7146	11352	6958	4800	12923	2022	20992	
6	E	24340	21679	20072	28434	19265	1309	4482	
7	F	19324	9525	345	14193	25169	13359	22855	
8	G	18191	6549	30899	14006	3144	14261	24631	
9	н	16447	31974	20447	25749	17469	14295	8583	
10									
11									
14 4	→ H\me	asurement/						<	

The export will be executed automatically if selected in the respective protocol file.



7.7.2 Data export via Export

- 1. Click on **Export** in the File menu
- 2. An EXCEL file will be created with file name resembling that of the data file

3. Open the *.xls file

The export will be executed	automatically if selected	in the respective protocol file.





💌 V	licrosoft Ex	cel - measur	ement.xls					
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3	В	85	15977	20362	15484	19795	10682	26103
4	С	11324	30983	24182	26153	5312	11992	18984
5	D	7146	11352	6958	4800	12923	2022	20992
6	E	24340	21679	20072	28434	19265	1309	4482
7	F	19324	9525	345	14193	25169	13359	22855
8	G	18191	6549	30899	14006	3144	14261	24631
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7.7.3 Direct data print-out

1. If not opened already open the respective data file by selecting **Open Data File** in the **File** menu

Eile	<u>V</u> iew	Instrume	nt <u>T</u> ools	<u>H</u> elp							
	New		Ctrl+	⊦N							
1	Open P	rotocol File	Ctrl-	+0	ewn	naster		Start Meas	urement 🔯	Protocol Pre	view 🔯 Re
	Save Pr	otocol File	Ctrl	+S				-			
	Save Pr	otocol File	<u>A</u> s		L						
	Open D	ata File				2	3	4	5	6	7
	Save Da	ata File ata File As.				11923	20347	19612	26973	31646	23681
a	Print		Ctrl	+P							
4	Print Pr Exit	e <u>v</u> iew				15977	20362	15484	19795	10682	26103
Ir	mmunoas	say	С	11324		30983	24182	26153	5312	11992	18984
	2111										

- 2. Select **Print Preview** in the **File** menu to get a preview of the print-out
- 3. Select **Print** in the **File** menu to start printing the data

					m	easur	ement. _{Data}	wgd					
A	22195	11923	3	4	8 28973	1 1946	7 23681	10207	34552	10	11	12 21292	
•	85	15977	21362	15464	19795	10552	26103	59 2	10853	12165	6225	25965	
c	11324	30983	24182	20103	8312	11992	15504	27103	27287	2740	18060	1918	
•	7148	11382	655	4800	12923	2122	2092	7188	28270	10383	24000	28128	
•	24540	21679	20072	28-04	19285	1309	46 	3789	32283	20237	17538	7565	
•	19224	9828	348	14193	25169	13389	22855	9044	18562	24085	22162	19801	
•	18191	84	30899	14008	3144	14281	24531	6338	411	21929	7239	30149	
-	16447	31974	22447	25740	17459	14225	8583	25202	877	7222	10719	22341	

The print-out will be executed automatically if selected in the respective protocol file.



8. Operation with Mikrowin 2000

Running measurements on the TriStar² is straight forward. The procedure is the same for all types of assay types, e.g. Raw Data, Dual Label, Kinetic, Repeated and Scanning. A measurement can be carried out immediately after a stored protocol is selected. At the end of each measurement the results are stored and may be printed or exported.

Result file names can be given without limitation. The extension is fixed, though. This is valid for measurement protocols as well.

8.1 Adding and Editing Microplate Dimensions

Microplates can differ in their dimensions dependent on brand and type. Please refer to the manufacturer's most recent information for exact dimensions of the microplates.

Microplates must be defined in the plate editor prior to defining a measurement protocol.

51. Click Plate Editor in the Instrument menu



52. Click **<New Plate>** or select a plate with matching well format and click **<Copy>**

Name		ОК
Berthold 96 - No:23300/23302	•	Cance
		New Pla
Number of rows (1 - 32):	8	Сору
Number of columns (1 - 48):	12	Apply
Height of the plate in (mm):	14.4	
Stacking height in (mm):	13.5	Remove F
Distance from corner and from	well to well in (mm)	
	14.38	
	+	
11.24		
	9	
	[a	



- 53. Assign a (descriptive) Name
- 54. Insert the **Number of rows**, e.g. **8** for a 96 well plate
- 55. Insert the Number of columns, e.g. **12** for a 96 well plate
- 56. Insert the total Height of the microplate

most 96 and 384 well plates are between 14 and 15.5 mm

57. Insert the **Stacking height** of the microplate

the stacking height is the resulting height (the visible part) when plates are put on top of each other (e.g. in a plate stacker)

in case this information is not available from the plate manufacturer the stacking height can be derived by stacking 2 plates and measuring the total height; by subtracting the regular height of one of the plates the resulting value will be the stacking height

- 58. Insert the distance between the left outer edge of the plate and the center of well A1
- 59. Insert the distance between to upper outer edge of the plate and the center of well A1
- 60. Insert the distance between the well centers of consecutive rows (vertical well distance)
- 61. Insert the distance between the well centers of consecutive columns (horizontal well distance)
- 62. Check the usage TriStar2

you may check additional instruments in case you have multiple instruments in operation

- 63. Click <Apply>
- 64. Click **<OK>**
- 65. The plate can now be used in the protocol files

Name		
Copy of Berthold 96 - No:23300.	/23302	Cance
Parameters		New Pla
Number of rows (1 · 32):	8	Сору
Number of columns (1 - 48):	12	Apply
Height of the plate in (mm):	14.4	Desserve
Stacking height in (mm):	13.5	
 Distance from corner and from 	well to well in (mm)	
11.24		
]a	



8.2 Single Raw Data measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine ATP content, single reporter gene expression, activities of caspases, kinases and many other enzymes.

8.2.1 Defining a Single Endpoint protocol

If you want to use an already existing protocol you may proceed with the next paragraph.

- 1. Click Read in the Options menu
- 2. Select BertholdTech TriStar2
- 3. Click <Settings>
- 4. Select the Plate Type (microplate format)

Note: the microplate has to be defined in the Plate Editor prior to defining a protocol

- 5. Define the **reading orientation**: by columns or by rows
- 6. Check **Meander** to have the instrument read one row from the left to right and the consecutive one from right to left or one row from top to bottom and the consecutive from bottom to top

	MikroWin 2 Deriving Data Reduction From its	MikroWin 2000 Deriving Data Reduction From its Roots	
river Selection		OK	
De <u>v</u> ice:	SertholdTech TriStar2	Cancel	
Version:	.01	Help	
Description:	eader device driver for Berthold	Settings	

Options 🗾
Samples Measurement Plate parameter Plate type: Berthold 96 - No.23300/23302 Plate Mode: Measurement Measurement Order: By Rows C By Columns Meander
1 2 3 4 5 6 7 8 9 1011 12 A O </th
OK Cancel Apply



- 7. Select the wells to be measured by clicking the Measurement radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells with a gray outside area are selected for measurement

Options 🗾
Samples Measurement Plate parameter Plate type: Berthold 96 - No 23300/23302 Plate Mode: Measurement Order: By Rows C By Columns Meander
1 2 3 4 5 6 7 8 9 10 11 12 A I
OK Cancel Apply

- Select the wells to be injected into and the respective injector by clicking the Inj 1, Inj 2 or Inj 3 radio button
 - for the whole plate, click the top left corner
 - for a row, click the respective character
 - for a column, click the respective number
 - for an area, click and drag the mouse
 - for an individual well, click into it

Wells coloured in the respective colour are injected into

Note: only wells to be measured can be injected into

Options X
Samples Measurement
Plate parameter Plate type: Berthold 96 - No:23300/23302 Plate Mode: C Measurement Order: © By Rows C Measurement Order: © By Rows C Measurement Order: © By Rows C By Columns Meander
1 2 3 4 5 6 7 8 9 10 11 12 A 3 4 5 6 7 8 9 10 11 12 B 3 4 5 6 7 8 9 10 11 12 C 3 4 5 6 7 8 9 10 11 12 C 3 4 5 6 7 8 9 10 11 12 B 3 5 6 7 8 9 10 11 12 C 4 5 6 7 8 9 10 11 12 C 7 8 9 10 10 10 C 7 8 9 10 10 10 C 7 8 9 10 10 C 7 8 9 10 10 C 7 8 9 10 10 10 10 C 7 8 9 10 10 10 10 10 10 10 10 10 10 10 10 10
OK Cancel Apply



9. Click onto the **Measurement** tab

Define the Measurement Operations

- Available operations are shown on the lefthand area
- Allowed operations are indicated by a check
 mark
- Double-clicking an operation open the specific properties dialogue
- Confirming the settings by clicking <OK> selects the operation and adds it to the operation sequence visible in the right-hand area
- The operation may be executed by plate the operation will be executed for all selected before the consecutive operation is started

by well all consecutive by well operations will be executed for a well before moving on to the next well

10. Double-click *Dispense* in case a reagent addition is required prior to the measurement

Injector	select 1, 2 or 3
Volume	10 to 100 µL
Speed	low – middle - high
Operation Mode	by plate or by well

11. Click **<OK>**

In case additional reagent additions are required repeat this procedure for the other injector(s)

- 12. Double-click *Delay* in case an delay/incubation time is required
 Duration 0.1 to 3600 s
 Operation Mode by plate or by well
- 13. Click **<OK>**

Measurement Sequence:	Operations:	Operation	
Ø Delay Shake Dispense Ounin. Label K Lumin. Knetic S Lumin. Scanning R Lumin. Repeated N Fuor. Label K Fluor. Knetic S Fluor. Scanning R Fluor. Repeated Absotbance K Abs. Knetic K Abs. Knetic T		<i>↑ ↓</i>	
Operation Mode Batch Plate Rep Temperature Control Temperature	25	°Celsius	

Parameters		OK
Injector:	1	Cancel
Volume:	10 💌	
Speed:	middle 👻	
Operation I	Mode: e]

Delay Properties	-		×
Duration:	3	(0.10 - 3600 s)	ОК
Operation Mode:	By plate	🔘 By well	Cancel
Perform on first plant	ate repeat only		


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14. Double-click Shake in case shaking is required

Duration	0.1 to 3600 s
Speed	slow, normal or fast
Diameter	0.1 to 5 mm
Туре	linear, orbital, double-orb.
Operation Mode	by plate or by well

15. Click <**OK**>

16.	Double-click <i>Lumin.L</i> reading	Label for a luminescence
	Name	give a (descriptive) name
	Counting Time	0.05 to 600 s
	Emission Filter	usually: No Filter
	<i>Note:</i> filters must be ment menu	defined prior in the Instru-
	Operation Mode	by plate or by well

- 17. Click <**OK**>
- 18. Double-click Fluor. Label for a fluorescence reading Name give a (descriptive) name **Counting Time** 0.05 to 600 s Lamp Energy 0 to 100 % select from the list **Excitation Filter**
 - **Emission Filter** select from the list
 - Note: filters must be defined prior in the Instrument menu Operation Mode by plate or by well
- 19. Click <**OK**>

Name

20. Double-click Absorbance for an absorbance reading

give a (descriptive) name 0.05 to 600 s Counting Time

0 to 100 % or **Auto** Lamp Energy

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter select from the list

Note: filters must be defined prior in the Instrument menu

Reference Measurement



Duration:	5	(0.10 - 3600 s)	ОК
Speed: Slow	Normal	🔘 Fast	Cance
Diameter:	2	(0.10 - 5 mm)	
Type: 🔘 Linear	Orbital	Double orbital	
Operation Mode:	By plate	By well	
Reform on first plate	repeat only		

Name:	luminescence 1	S	ОК
Counting Time:	1	(0.05 - 600 s)	Cancel
Emission Filter:	No Filter - Slot A	•	
Operation Mode:	By plate	O By well	
Second Measurer	ment		
Emission Filter	No Filter - Slot	45 -	

Name:	Fluorescence485/535	ОК
Counting Time:	0.10 (0.05 - 600 s)	Cancel
Lamp Energy:	35	(-)
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2	
Emission Filter:	F535 (FITC Fluorescein) - Slot A2	
	ment	
Second Measure		
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2 *	

orbance		1.01 000	
Name:	absorbance45	50	ОК
Counting Time:	0.10	(0.05 - 600 s)	Cancel
Lamp Energy:	Auto		Auto
Measurement <mark>Filter:</mark>	F450 (Absorba	ance) - Slot A1 🔹	
Reference Measur	ement		
Reference Filter:	F450 (Absorba	ance) - Slot A1 🔹	

Note: the values derived with this filter will be automatically subtracted from the measurement value per well Reference Filter select from the list

Operation Mode by plate or by well

21. Click **<OK>**

22. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

23. Check **Batch** and define the number of plates in **Plate Repeats** in case you want a number of plates to be stored into a single data file

Note: this setting can only be used in single endpoint measurements

- 24. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed
- 25. Check **Temperature** to activate the temperature control for this protocol
- 26. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 27. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 28. Click **<OK>**

29. Click <OK> once more



leasurement Sequence:	Operations:	Operation	
✓ Delay [✓ Shake [✓ Dispense [✓ Lumin. Label ✓ K Lumin. Knetic □ R Lumin. Repeated ✓ Fluor. Kinetic □ S Fluor. Scanning □ R Lumin. Kinetic □ S Fluor. Scanning □ R Fluor. Repeated ✓ Absochance ✓ K Abs. Kinetic III	↓ Dispense ①Delay ③ Table ③ Shake ④ Wumin Label	Name Counting Time Meas. operation	Lumin. Label 0.10 by Well
Operation Mode Batch Plate Repe Temperature Control	ats: 1	\$	
- K N			

30. By default the plain measurement data will be located on **Result matrix 2** "**Reader_Values**"

For export und print you have to refer to this matrix.

Should you wish to define any additional calculations please refer to the Mikrowin manual.

- 31. To activate automatic export click **Export Setup...** in the **File** menu
- 32. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

- 33. Click **<OK>**
- 34. Click **Print Setup...** in the File menu
- 35. Select the data set by highlighting and clicking <**Add>**

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
Reader_Values	measured data (matrix 2)*
Results	Averages (matrix 3)*
Gen. Statistics	measurement settings

* the selection and content depends on the matrix definition done in the Calculation section

- 36. Check if Automatic Print-out is required
- 37. Type a header and/or footer



Deriving Data Redu	ction From its Roots	
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- 39. Go to File | Save As...
- 40. Create and/or Select an appropriate directory, e.g. *ParaTriStar2*
- 41. Select the file type Mikrowin Para File (*.par)
- 42. Type a meaningful file name
- 43. Click <Save>

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Recent Places	e testtraining	3.par	02/28/2011 16:51	PAR File
INCLINUIK	•	111		
	File name:		•	Save
	Save as type:	MikroWin Para File (*.par)	•	Cancel



SX 1

8.2.2 Measurement with a Single Endpoint protocol

The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

0----

Note: Make sure the appropriate plate frame is inserted

- 1. Click **Open** in the **File** menu
- 2. Select File of type: Mikrowin Para File
- 3. Select the appropriate file
- 4. Click <Open>

5.	Enter a file name under which the measurement
	is to be stored

Look in:	🔑 Para Tri Star2	💌 🔶 🖻 👘 💷 •	
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Recent Places	🖻 singlelumiwithinj.par	06/27/2012 10:59	PAR File
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	File name:	•	Open
	Files of type: MikroWin Para File	(* par)	Cancel

- Martin				
MikroWin 2000				
Deriving Data Reduction	1 San	nple_ID	Reader_Values	Besults
From its Roots		File manage Inc.	as todau	Cishin Cishin

- 6. Click <Start>
- 7. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (±1 mm), e.g. 6, 12, 24 well plates

8. Click <OK>



Start



 The selected wells of the microplate will be measured and the numerical value of the signal will be displayed



10. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument





8.3 Dual Label Assay measurement

A raw data measurement generates pure RLU (or RLU/s) values for each measured well. This measurement type is useful in luminescent research assays to determine dual reporter gene expression.

8.3.1 Defining a Dual Label protocol

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click *Lumin.Label* for a luminescence

reading	
Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Emission Filter	usually: No Filter
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Operation Mode	by plate or by well

17. Double-click *Lumin.Label* once more and define the settings for the second reading

For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

Name:	luminescence 1s	1	ОК
Counting Time:	1	(0.05 - 600 s)	Cancel
Emission Filter:	No Filter - Slot A	5 🔹	
Operation Mode:	By plate	O By well	
Second Measurer	ment		
Emission Filter:	No Filter - Slot A	5 -	



19. Double-click *Fluor. Label* for a fluorescence reading
Name give a (descriptive) name Counting Time 0.05 to 600 s
Lamp Energy 0 to 100 %
Excitation Filter select from the list Emission Filter

Note: filters must be defined prior in the Instrument menu

Operation Mode by plate or by well

20. Double-click *Fluor.Label* once more and define the settings for the second reading

For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

- 21. Click **<OK>**
- 22. Double-click **Absorbance** for an absorbance reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or <i>Auto</i>

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Excitation Filter select from the list

Note: filters must be defined prior in the Instrument menu

Reference Measurement

Note: the values derived with this filter will be automatically subtracted from the measurement value per well

Reference Filter	select from the list
Operation Mode	by plate or by well

23. Double-click *Absorbance* once more and define the settings for the second reading

For very fast switch between the first and the second reading, e.g. for fast BRET kinetics or for



Name:	Fluorescence485/535	ОК
Counting Time:	0.10 (0.05 - 600 s)	Cancel
Lamp Energy:	35	<u>()</u>
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2	
Emission Filter:	F535 (FITC Fluorescein) - Slot A2	
Second Measuren	ient	
Excitation Filter:	F485 (FITC Fluorescein) - Slot A2 🔹	
Emission Filter:	F535 (FITC Fluorescein) - Slot A2 *	

	absorbance45	0	ОК
Counting Time:	0.10	(0.05 - 600 s)	Cancel
Lamp Energy:	Auto	· · · · · · ·	Auto
Measurement Filter:	F450 (Absorba	nce) - Slot A1 🔹	
🔲 Reference Measur	rement		
Reference Measur	ement F450 (Absorbar	nce) - Slot A1 👻	

ratiometric readings monitoring fast reaction kinetics you may check **Second Measurement** instead of defining a second label

You may define additional operations, e.g. Dispense, Delay or Shaking in between the two measurement operations, e.g. in DLR assays

24. Click **<OK>**

25. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

26. Do not check Batch

Note: this setting can only be used in single endpoint measurements

- 27. Define a number in **Plate Repeats** only in case you want the selected operations to be repeatedly executed
- 28. Check **Temperature** to activate the temperature control for this protocol
- 29. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 30. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 31. Click **<OK>**
- 32. Click **<OK>** once more



o Contraction of the sequence.	perations:	Operation	
Y Stableay [Y Stake [Y Ispense [Y Lumin, Label [Y Lumin, Kinetic [S Lumin, Scanning [R Lumin, Repeated [Y Ruor, Label [Y K Huor, Kinetic [S Ruor, Scanning [R Ruor, Roeated [Y Absothance [Y K Abs. Kinetic [Y Absothance [Y Muset [★ Dispense ♦ Dispense ♥ Delay ₩ Shake ₩ Lumin, Label 	Name Counting Time Meas. operation	Lumin. Label 0.10 by Well
peration Mode Batch Plate Repea emperature Control Temperature	ts: 1 25 *Celsiu	8	

For further calculations of the measurements follow the next steps:

- 33. Go to **Options | Matrix** and click **<Add Matrix>**
- 34. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **first reading**

Do the same for Matrix No. 3 (rename to e.g. **second reading**) and Matrix No. 4 (rename to e.g. **ratio**)

35. Click **<OK>**

For export und print you have to refer to this matrices.

- 36. Change the view to the Calculation section
- 37. Click on the 2 first reading tab
- 38. Type **LB1** into the Calculation Formula: LB1 = Label 1 = first of readings
- 39. Click the double-cross to assign for all wells



40. Proceed with the two other matrices alike:

3 second reading LB2

LB 2 = Label 2 = second of readings

4 ratio MA2/MA3 MA2 = Matrix 2, MA3 = Matrix 3, MA2/MA3 = values of Matrix 2 divided by values of Matrix 3

Should you wish to define any additional calculations please refer to the Mikrowin manual.





] 2	first 3] <u>3</u> seco	and 4 ratio1	/2
I	LB1	LB2	MA2/MA3	1
i	LB1	LB2	MA2/MA3	ł
	LB1	LB2	MA2/MA3	,



- 41. To activate automatic export click **Export Setup...** in the **File** menu
- 42. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

- 43. Click **<OK>**
- 44. Click **Print Setup...** in the File menu
- 45. Select the data set by highlighting and clicking

<add></add>	
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
first	measured data (matrix 2)*
second	measured data (matrix 3)*
ratio	ratio of readings (matrix 3)*
Gen. Statistics	measurement settings

* the selection and content depends on the matrix definition done in the Calculation section

46. Check if Automatic Print-out is required

47. Type a header and/or footer

File Export Setup MikroWin 2000 Deriving Data R n Fro List of Active Export Driver OK List of export driver Ŧ Cancel List of export driver • III Help Line Export Driver RawData Export Driver Graphic Export Driver 4 Remove

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- 49. Go to File | Save As...
- 50. Create and/or Select an appropriate directory, e.g. *ParaTriStar2*
- 51. Select the file type Mikrowin Para File (*.par)
- 52. Type a meaningful file name
- 53. Click <Save>

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Recent Places	e testtraining	j.par	02/28/2011 16:51	PAR File
Network		111		
	File name:		_	Save
	Save as type:	MikroWin Para File (*.par)	•	Cancel



8.3.2 Measurement with a Dual Label Assay protocol

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.
- Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2** "**Measurement with a single endpoint rotocol**".



8.4 Kinetic Measurement

A kinetic measurement mode is appropriate for fast kinetics assays lasting over several seconds up to minutes, e.g. enzyme kinetics and Calcium influx

8.4.1 Defining a protocol for a kinetic measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click *Lumin. Kinetic* for a luminescence kinetic reading

•	
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake ins	stead of Delay if needed
Delay	0 to 600 sec
Repeats	(are calculated)
Emission Filter	usually: No Filter

Note: filters must be defined prior in the Instrument menu

Second Measurement may be checked in case of ratiometric kinetics, e.g. in BRET applications

17. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

Name: LumiKnetc OK Total Time: 10.00 (1 - 604800 s) Cancel Counting Time: 1.00 (0.05 - 600 s) Cancel Use Shake instead of Delay Delay: 0.00 (0 - 600 s) Repeats: 11 (1 - 50000) Emission Filter: No Filter - Slot A5 Ensistion Filter: No Filter - Slot A5 • •	netics			×
Total Time: 10.00 (1 - 604800 s) Cancel Counting Time: 1.00 (0.05 - 600 s) Cancel Use Shake instead of Delay Delay: 0.00 (0 - 600 s) Repeats: 11 (1 - 50000) Cancel	Name:	LumiKinetic		ОК
Counting Time: 1.00 (0.05 - 600 s) Use Shake instead of Delay Delay: 0.00 (0 - 600 s) Repeats: 11 (1 - 50000) Emission Filter: No Filter - Slot A5 Emission Filter: No Filter - Slot A5 ▼	Total Time:	10.00	(1 - 604800 s)	Cancel
Use Shake instead of Delay Delay: 0.00 (0 - 600 s) Repeats: 11 (1 - 50000) Emission Filter: No Filter - Slot A5 Emission Filter: No Filter - Slot A5	Counting Time:	1.00	(0.05 - 600 s)	
Delay: 0.0 (0 - 600 s) Repeats: 11 (1 - 50000) Emission Filter: No Filter - Slot A5 Filter - Slot A5 Second Measurement Emission Filter: No Filter - Slot A5 The state of the st	🔲 Use Shake inste	ad of Delay		
Repeats: 11 (1 - 50000) Emission Filter: No Filter - Slot A5 • Second Measurement Emission Filter: No Filter - Slot A5 •	Delay:	0.00	(0 - 600 s)	
Emission Filter: No Filter - Slot A5 Second Measurement Emission Filter: No Filter - Slot A5	Repeats:	11	(1 - 50000)	
Second Measurement Emission Filter: No Filter - Slot A5	Emission Filter:	No Filter - Slot	A5 🔹	
Second Measurement Emission Filter: No Filter - Slot A5				
Emission Filter: No Filter - Slot A5	Second Measure	ement		
	Emission Filter:	No Filter - Slo	t A5 👻	



18. Double-click *Fluor. Kinetic* for a fluorescence kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Check Use Shake ins	stead of Delay if needed
Delay	0 to 600 s
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
Note filters must be	defined prior in the Instru-

Note: filters must be defined prior in the Instrument menu

Operation Mode by plate or by well

Second Measurement may be checked in case of ratiometric kinetics, e.g. in Calcium applications

19. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way

20. Double-click **Abs. Kinetic** for an absorbance kinetic reading

Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)

Counting Time 0.05 to 600 s

Check Use Shake instead of Delay if needed

Delay 0 to 600 s

Repeats (are calculated)

Lamp Energy 0 to 100 % or *Auto*

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Note: filters must be defined prior in the Instrument menu

21. Click **<OK>**

a second or third kinetic operation may be added, e.g. after a dispensing operation, and set up in the same way



Name:	FluoKinetic		ОК
Fotal Time:	5	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Use Shake inste	ad of Delay		
Delay:	0.00	(0 - 600 s)	
Repeats:	9	(1 - 50000)	
Lamp Energy:	100	an Alakara ara ara	- O
Lamp Energy: Excitation Filter:	100 F485 (FITC F	luorescein) - Slot A2	Q
Lamp Energy: Excitation Filter: Emission Filter:	100 F485 (FITC FI F535 (FITC FI	luorescein) - Slot A2	. Ģ
Lamp Energy: Excitation Filter: Emission Filter: I Second Measure	100 F485 (FITC R F535 (FITC R	luorescein) - Slot A2	Ţ
Lamp Energy: Excitation Filter: Emission Filter: I Second Measure Excitation Filter:	100 F485 (FITC R F535 (FITC R ment F485 (FITC R	luorescein) - Slot A2	

Name:	Kinetic		ОК
Total Time:	20	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Use Shake instead	of Delay		
Delay:	1	(0 - 600 s)	
Repeats:	19	(1 - 50000)	
Lamp Energy:	Auto	<u>, , , , , , , , , , , , , , , , , , , </u>	📝 Auto
Measurement Filter:	F450 (Absorb	ance) - Slot A1 🔹	

22. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

- 23. Check **Temperature** to activate the temperature control for this protocol
- 24. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 25. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 26. Click **<OK>**
- 27. Click <OK> once more

For further calculations of the measurements follow the next steps:

- 28. Go to Options | Matrix and click <Add Matrix>
- 29. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)

30. Click **<OK>**

For export und print you have to refer to these matrices.



Derivin	MikroWin 20 og Data Reduction From its Ro	00 oots
eets Direction		
Matrix Name Sample_ID AUC (Integral) Vmax Peak	Matrix Number Matrix No. 1, (MA1) Matrix No. 2, (MA2) Matrix No. 3, (MA3) Matrix No. 4, (MA4)	Add Matrix
		Edit Matrix



OK

Cancel

Help

MikroWin 2000

Deriving Data

Variance of kinetics delta values 🌛

Deviation of kinetics delta values

Coefficient of variation of kinetics

Minimum of kinetics measurement

Maximum of kinetics measurement

Time of kinetics minimum

Time of kinetics maximum

Kinetics time of maximal slope

Delta per minute for selected range

Delta correlation for selected range

Delta per minute at maximal slope

Delta correlation at maximal slope

Kinetics onset time

Add Formula

KDWV(MEA)

KDWD(MEA)

KDWC(MEA)

KMIN(MEA)

KMAX(MEA)

KTMI(MEA)

KTMA(MEA)

KTDO(MEA)

KTMS(MEA)

KDMR(MEA)

KRVR(MEA)

KDMS(MEA)

KRVS(MEA)

AUC (Integral)

- 31. Change the view to the **Calculation** section
- 32. Click on the 2 AUC (Integral) tab
- 33. Click <Add Formula> and expand Kinetic calculation functions
- 34. Select KITG(MEA) and click <OK>
- 35. Click the double-cross to assign for all wells



- 36. Proceed with the two other matrices alike:
 - **3** Vmax

KDMS(MEA)

4 Peak

KMAX(MEA)

When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.

- 37. To activate automatic export click **Export Set**up... in the **File** menu
- 38. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.



3 Vmax

4 Pe

4 Peak

- 39. Click **<OK>**
- 40. Click **Print Setup...** in the File menu
- 41. Select the data set by highlighting and clicking

header part
par and dat files names
raw data
sample info (matrix1)*
area under curve (matrix 2)*
delta @ max slope (ma. 3)*
peak value (matrix 4)*





KSLP(MEA) Slope of regression line of kinetic r KTSP(MEA) Length of time of the selected ran					
KITG(MEA)	KITG(M	MEA)	KDMS(MEA)	(MEA)	KMAX(MEA)
KITG(MEA)	KITG(M	MEA)	KDMS(MEA)	(MEA)	KMAX(MEA)

Gen. Statisticsmeasurement settingsKinetics Graphicscurves

* the selection and content depends on the matrix definition done in the Calculation section

- 42. Check if Automatic Print-out is required
- 43. Type a header and/or footer



- 45. Go to File | Save As...
- 46. Create and/or Select an appropriate directory, e.g. *ParaTriStar2*
- 47. Select the file type Mikrowin Para File (*.par)
- 48. Type a meaningful file name
- 49. Click <Save>

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Save in	: 🔒 Para	<u>-</u>	• 🖬 🖆 🐨	
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Network		111		
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	ring righter.	1		0.010



8.4.2 Kinetic measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.
- Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2** "**Measurement with a single endpoint rotocol**".



8.5 Repeated Measurement

A repeated measurement mode is appropriate for long-term kinetic assays lasting over multiple minutes up to several days, e.g. cellular luminescence, slow enzyme kinetics, long-term gene expression or growth monitoring

8.5.1 Defining a protocol for a repeated measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click *Lumin. Repeated* for a luminescence repeated reading

Name Total Time	give a (descriptive) name the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Emission Filter	usually: No Filter
Counting Time Cycle Time Repeats Emission Filter	7 days) 0.05 to 600 s the time a specific well is read again in the consecu- tive cycle (are calculated) usually: No Filter

Note: filters must be defined prior in the Instrument menu

Injector 1, ...2, ...3

Check Use Injector for an injection within the re-

	peated cycle
Injector Cycle	<i>0</i> means prior to a meas- urement
Volume	10 to 100 μL
Speed	1 to 5
Operation Mode	by plate or by well

17. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (BRET)

peated	-	- No. Sector	— X
Name:	LumiRepeated		ОК
Total Time:	3600.00	(1 - 604800 s)	Cancel
Counting Time:	1.00	(0.05 - 600 s)	
Cycle Time:	124.14	(124.14 - 6000 s)	
Repeats:	29	(1 - 50000)	
Emission Filter:	No Filter - Slot	A5 👻	
Use Injector	4	(0 - 29)	
Volume:	100		
Speed:	2	_	



18. Double-click *Fluor. Repeated* in the Fluorescence section for a fluorescence repeated read-

ing	
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
<i>Note:</i> filters must be ment menu	defined prior in the Instru-

Injector 1, ...2, ...3

Check Use Injector for an injection within the re-

poulou oyolo
0 means prior to a meas- urement
10 to 100 µL
1 to 5
by plate or by well

Fluorescence Repeated Fluo Repeated 1 ОК Name: Total Time: 300.00 (1 - 604800 s) Cancel Counting Time: 0.10 (0.05 - 600 s) Cycle Time: 33.33 (33.33 - 6000 s) Repeats: 10 (1 - 50000) 0 Lamp Energy: 100 Excitation Filter: F485 (FITC Fluorescein) - Slot A2 Emission Filter: F535 (FITC Fluorescein) - Slot A2 🔹 Injector 1 Injector 2 Injector 3 Use Injector 0 Injector Cycle: (0 - 10)100 Volume: 2 Speed: Operation Mode: By plate By well

19. Click **<OK>**

a second repeated operation may be added, e.g. for ratiometric applications (FRET)



20. Double-click *Abs. Repeated* in the Absorbance section for a absorbance repeated reading

	· •
Name	give a (descriptive) name
Total Time	the entire kinetic time (max. 7 days)
Counting Time	0.05 to 600 s
Cycle Time	the time a specific well is read again in the consecu- tive cycle
Repeats	(are calculated)
Lamp Energy	0 to 100 % or <i>Auto</i>

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Check Reference Measurement if needed

Reference Filter select from the list

Note: filters must be defined prior in the Instrument menu

Injector 1, ...2, ...3

Check Use Injector for an injection within the re-

	poulou oyolo
Injector Cycle	0 means prior to a meas- urement
Volume	10 to 100 µL
Speed	1 to 5
Operation Mode	by plate or by well

21. Click <OK>

a second repeated operation may be added, e.g. for ratiometric applications

	AlenDerester		OK
Name:	Abshepealed		
Total Time:	600	(1 - 604800 s)	Cancel
Counting Time:	0.10	(0.05 - 600 s)	
Cycle Time:	60	(35.29 - 6000 s)	
Repeats:	11	(1 - 50000)	
.amp Energy:	Auto	<u>,</u> Q	🔽 Auto
Measurement Filter:	F450 (Absorb	ance) - Slot A1 🔹	
Reference Measur	ement F450 (Absorb)	ance) - Slot A1 🔹	
Reference Measure	ement F450 (Absorb:	ance) - Slot A1 v	
Reference Measure Reference Filter: Injector 1 Injector 2	F450 (Absorb	ance) - Slot A1 🔹 👻	
Reference Measure Reference Filter: Injector 1 Injector 2 V Use Injector	F450 (Absorb	ance) - Slot A1 *	
Reference Measure Reference Filter: Injector 1 Injector 2 Use Injector Injector Cycle:	F450 (Absorb)	ance) - Slot A1 *	
Reference Measure Reference Filter: Injector 1 Injector 2 V Use Injector Injector Cycle: Volume:	ement F450 (Absorb: Injector 3 0 100	ance) - Slot A1 *	
Reference Measure Reference Filter: Injector 1 Injector 2 V Use Injector Injector Cycle: Volume: Speed:	ement F450 (Absorb: Injector 3 0 100 2	ance) - Slot A1 (0 - 11)	



22. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

- 23. Check **Temperature** to activate the temperature control for this protocol
- 24. Define the target temperature

the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 25. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 26. Click **<OK>**
- 27. Click <OK> once more

For further calculations of the measurements follow the next steps:

- 28. Go to Options | Matrix and click <Add Matrix>
- 29. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **AUC (Integral)**

Do the same for Matrix No. 3 (rename to e.g. **Vmax**) and Matrix No. 4 (rename to e.g. **Peak**)

30. Click **<OK>**

For export und print you have to refer to these matrices.

Measurement Sequence:	Operations:	Operation	
Image: State of the state	R LumiRepeated	Name Counting Time Meas.operation Emission Filter Cycle Time Total Time Injector Cycle Volume Speed	LumiRepeated 0.10 by Well No Filter 60.00 300.0 2 0 10 10 low
Operation Mode Batch Plate Repeat Temperature Control Temperature Keep Plate after Meas.	sts: 6	3	

Der	MikroWin 20	00 oots
neets Direction		
ist of defined Matrices Matrix Name Sample_ID	Matrix Number Matrix No. 1, (MA1)	
AUC (Integral) Vmax Peak	Matrix No. 2, (MA2) Matrix No. 3, (MA3) Matrix No. 4, (MA4)	Add Matrix
rouk	House Ho. 4, (MAH)	Edit Matrix



OK

Cancel

Help

MikroWin 2000

Deriving Data

Variance of kinetics delta values 🌛

Deviation of kinetics delta values

Coefficient of variation of kinetics

Minimum of kinetics measurement

Maximum of kinetics measurement

Time of kinetics minimum

Time of kinetics maximum

Kinetics time of maximal slope

Delta per minute for selected range

Delta correlation for selected range

Delta per minute at maximal slope

Kinetics onset time

- 31. Change the view to the Calculation section
- 32. Click on the **2** AUC (Integral) tab
- 33. Click <Add Formula> and expand Kinetic calculation functions
- 34. Select KITG(MEA) and click <OK>
- 35. Click the double-cross to assign for all wells



- 36. Proceed with the two other matrices alike:
 - **3** Vmax

KDMS(MEA)

4 Peak

KMAX(MEA)

When 2nd measurement has been checked MEA need to be replaced by LB1 and LB2 respectively and additional matrices may need to be created

Should you wish to define any additional calculations please refer to the Mikrowin manual.

- 37. To activate automatic export click Export Setup... in the File menu
- 38. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.



3 Vmax 🛛

4 Pe

4 Peak

- 39. Click <**OK**>
- 40. Click Print Setup... in the File menu
- 41. Select the data set by highlighting and clicking ~Add>

<auu></auu>	
Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
AUC	area under curve (matrix 2)*
Vmax	delta @ max slope (ma. 3)*
Peak	peak value (matrix 4)*





KITSPIM	EA) Deita EA) Integ EA) Slope EA) Leng III	correlation ral of kinet of regress	at maximal stope ics measurement ion line of kinetic r if the selected rank		
KITG(MEA)	KITG(M	MEA)	KDMS(MEA)	(MEA)	КМАХ(МЕА)
KITG(MEA)	КІТС(М	MEA)	KDMS(MEA)	(MEA)	KMAX(MEA)

Add Formula

KDWV(MEA)

KDWD(MEA)

KDWC(MEA)

KMIN(MEA)

KMAX(MEA)

KTMI(MEA)

KTMA(MEA)

KTDO(MEA)

KTMS(MEA)

KDMR(MEA)

KRVR(MEA)

KDMS(MEA)

AUC (Integral)

Gen. Statisticsmeasurement settingsKinetics Graphicscurves

* the selection and content depends on the matrix definition done in the Calculation section

- 42. Check if Automatic Print-out is required
- 43. Type a header and/or footer



- 45. Go to File | Save As...
- 46. Create and/or Select an appropriate directory, e.g. *ParaTriStar2*
- 47. Select the file type Mikrowin Para File (*.par)
- 48. Type a meaningful file name
- 49. Click <Save>

Save As					×
Save in:	🔒 Para		-	← 🗈 💣 💷 ◄	
C.	Name	*		Date modified	Туре
Recent Places	etesttraining	j.par		02/28/2011 16:51	PAR File
Hochone	•				Þ
	File name:			•	Save
	Save as type:	MikroWin Para File (*.par)		•	Cancel



8.5.2 Repeated measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.
- Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2** "**Measurement with a single endpoint rotocol**".



8.6 Scanning Measurement

A scanning measurement mode is appropriate for assays with heterogeneous distribution of signal, e.g. cellular assays

8.6.1 Defining a protocol for a scanning measurement

Follow the instructions until step 15 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

16. Double-click *Fluor. Scanning* for a fluorescence scanning reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 %
Excitation Filter	select from the list
Emission Filter	select from the list
<i>Note:</i> filters must be ment menu	defined prior in the Instru-
Steps	1 to 100 scanning points in one di- rection, the other direction will have the same amount of points

uorescence Scanning			×
Name:	ScanningFluo		ОК
Counting Time:	0.10	(0.05 - 600 s)	Cancel
Lamp Energy:	10		
Excitation Filter:	F485 (FITC Fluor	escein) - Slot A2	
Emission Filter:	F535 (FITC Fluor	escein) - Slot A2 🔹	
Steps:	3	(1 - 100)	
Point Displacement:	2.5	(0.09 - 50 mm)	
Scanning Mode:	Rectangular	Round	

Point Displacement distance between points Select rectangular or round matrix

- 17. Click **<OK>**
- 18. Double-click *Abs. Scanning* for a absorbance scanning reading

Name	give a (descriptive) name
Counting Time	0.05 to 600 s
Lamp Energy	0 to 100 % or Auto

Note: Auto is recommended; it uses the calibrated energy setting specific for the selected filter

Measurement Filter select from the list

Note: filters must be defined prior in the Instrument menu

Steps	1 to 100
	scanning points in one di-
	rection, the other direction
	will have the same amount
	of points

Point Displacement distance between points





Select rectangular or round matrix

- 19. Click **<OK>**
- 20. The **sequence of selected operations** will be displayed in the center column

Operations can be moved up or down by highlighting the operation and clicking on the respective arrow

Operations can be deleted by highlighting and clicking the cross

Details of the operation highlighted can be viewed on the right column

An operation can be edited by double-clicking on it in the center column

- 21. Check **Temperature** to activate the temperature control for this protocol
- 22. Define the **target temperature** the instrument will start to heat the plate compartment as soon as the protocol file will be loaded

Robot, Barcode and Multi Plate Data File Mode are currently not active

- 23. Check **Keep Plate after Measurement** in case you want the microplate being kept inside the instrument after the reading being finished
- 24. Click **<OK>**
- 25. Click <OK> once more

For further calculations of the measurements follow the next steps:

- 26. Go to **Options | Matrix** and click **<Add Matrix>**, click **<Add Matrix>** again
- 27. Highlight Matrix No. 2, click **<Edit Matrix>** and rename to e.g. **total**

Do the same for Matrix No. 3 (rename to e.g. **max**), Matrix No. 4 (rename to e.g. **min**) and Matrix No. 5 (rename to e.g. **average**)

28. Click **<OK>**

For export und print you have to refer to these matrices.



Medsulement Jequence.	Operations:		Operation	
	¥ 1 S RuoScan	* 4	Name Counting Time Lamp Energy [%] Excitation Filter Horizontal steps Vertical steps Point displacement Scanning mode Meas. operation	FluoScan 0.10 8 F485 F460 10 10 0.56 Rectangular by Well
Operation Mode Batch Plate Repe Temperature Control	ats: 1	Celsius	• [•

Der	MikroWin 20 iving Data Reduction From its Re	oots	
eets Direction			
ist of defined Matrices Matrix Name	Matrix Number		
Sample_ID	Matrix No. 1, (MA1) Matrix No. 2 (MA2)		
max	Matrix No. 3, (MA3)	Add Matrix	
average	Matrix No. 5, (MA5)	Edit Matrix	

- 29. Change the view to the Calculation section
- 30. Click on the 2 total tab
- 31. Click <Add Formula> and expand Area Scan functions
- 32. Select SNUM(MEA) and click <OK>
- 33. Type an asterisk (*)
- 34. Click <Add Formula> and expand Area Scan functions
- 35. Select SAVE(MEA) and click <OK>

Calculation Formula of Position A01 : SNUM(MEA)*SAVE(MEA)

Add Formula MikroWin 2000 Deriving Data I E Statistic functions using free partition ΠK + Statistic functions using control overlay Cancel Threshold calculation functions Help - Curvefit calculation functions E- Kinetic calculation functions Hultiplate calculation functions - Agglutination calculation functions . WavelengthScan calculation functions SMIN(MEA) Minimum of AreaScan measureme SMAX(MEA) Maximum of AreaScan measuremei SAVE(MEA) Average of AreaScan measureme SNUM(MEA) Number of AreaScan measuremen SSD(MEA) Standard Deviation of AreaScan me SCV(MEA) Coefficient of variation of AreaScan Spacial calculation functions

3 max / 4 min

5 average

 EA)
 ...)"SAVE(MEA)
 SMAX(MEA)
 SMIN(MEA)
 SAVE(MEA)

 EA)
 ...)"SAVE(MEA)
 SMAX(MEA)
 SMIN(MEA)
 SAVE(MEA)

2 total 3 ma

36. Click the double-cross to assign for all wells



37. Proceed with the three other matrices alike:

3 max SMAX(MEA)

4 min SMIN(MEA)

5 average SAVE(MEA)

Should you wish to define any additional calculations please refer to the Mikrowin manual.

- 38. To activate automatic export click **Export Set**up... in the **File** menu
- 39. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

Deriving Data Rec	kroWin 2000	
ist of Active Export Driver		OK
List of export driver	-	Cancel
List of export driver Line Export Driver		Help
Matrix Export Driver RawData Export Driver		
Graphic Export Driver	*	



- 41. Click Print Setup... in the File menu
- 42. Select the data set by highlighting and clicking <**Add>**

Page Header	header part
File Names	par and dat files names
Sample ID	sample info (matrix1)*
total	sum of readings (matrix 2)*
max	max. reading (matrix 3)*
min	min. reading (matrix 4)*
average	average of read. (matrix 5)*
Gen. Statistics	measurement settings
Scanning Graphics	blue-to-red map

* the selection and content depends on the matrix definition done in the Calculation section

- 43. Check if Automatic Print-out is required
- 44. Type a header and/or footer

	Deriving	Data Red	uction F	rom its Roots	- Contraction
ompilation Automation	Header Man	oins			
Avaiable Items			Selecte	ed Items	
User statistics 2 User statistics 3 Bar Graphics Color Graphics Curvefit Graphics Kinetics Graphics	^	⇒	Pag File Sam total max min	e Header Names ple_ID < Matrix < Matrix > < Matrix > < Matrix >	0
Selection Graphics Control Graphics New Page Separator	E .		Gen Sca	age (Matrix) eral statistics nning Graphics	
Format :	*		(Add	Remove
	ОК	Car	ncel	Printer	Н
		_			_
Print Setup	-	-		-	-
		D.41	krolA	lin 2000	-
	Deriving	Data Red	uction Fi	rom its Roots	- Carlos
Automatic printou	t after measuren	ient			
Automatic printou	t after measuren	rent			
Automatic printou	t after measuren	nent	ncel	Printer	н
Iv Automatic printou Print Setup	t after measuren	ent Car	ncel	Printer	В
✓ Automatic printou Print Setup	OK Devices	Car Mil	ncel	Printer	<u> </u>
Automatic printou Print Setup Compilation Automation	OK Deriving Header Mar	Car Mil Data Red	Incel	Printer Fin 2000 orm its Roots	н
Automatic printou Print Setup Compilation Automation Joacker	OK Deriving Header Mar	Car Mil Data Red gins	ncel	Printer Fin 2000 orm its Roots	<u> </u>
Automatic printou Print Setup Implation Automation Header	OK Deriving Header Mar	Car Mil g Data Red gins	ncel	Printer Fin 2000 om its Roots	<u> </u>
Automatic printou Print Setup implation Automation Header	OK Deriving Header Mar	Car Mil 1 Data Red gins	kroW	Printer	<u>н</u>
Automatic printou Print Setup Compilation Automation Header Header Fort : Aria	OK Deriving Header Mar	Car Mill 1) Data Red	Incel	Pinter	H
Automatic printou Print Setup Compilation Automation Header Header Font : Aria Footer	OK Deriving Header Mar	Car Mil gins	kroW	Pinter Fin 2000 com its Roots	Bet Fort
Automatic printou Automatic printou Print Setup Implation Automation Header Header Font : Aria Header Font : Aria	OK Deriving Header Mar	Car Mili grs	kroW uction Fi	Printer Tin 2000 om its Roots	Bet Font
Automatic printou Print Setup Compilation Automation Header Header Font : Aria Footer Foo	OK Deriving Header Mar	Ca Mili Data Red	kroW	Printer Tim 2000 com its Roots	Set Font
Automatic printou Automatic printou Print Setup Iompilation Automation Header Header Font : Ana Footer Footer Foot	OK Deriving Header Mar	Car Car Data Red	kcel	Printer Tin 2000 orm its Roots	Set Fort

File Print Setun

- 46. Go to File | Save As...
- 47. Create and/or Select an appropriate directory, e.g. **ParaTriStar2**
- 48. Select the file type Mikrowin Para File (*.par)
- 49. Type a meaningful file name
- 50. Click <Save>





8.6.2 Scanning measurement

The protocol that has been created will be pre-selected. In case you want to perform a measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.
- Note: Make sure the appropriate plate frame is inserted

Follow the steps as described in paragraph **8.2.2** "**Measurement with a single endpoint rotocol**".



8.7 Measurement with curve fitting

A raw data measurement that contains standards with known concentrations which are used to determine unknown concentrations of the samples.

8.7.1 Defining a protocol with curve fitting

When working with Blank subtraction it is recommended to change a global setting in Mikrowin first.

- 1. Go to **Installation | Settings** and hit the **ALT** and the **S** keys
- 2. Scroll to 014 DisableAutoBlankSubtraction
- 3. Select the value Yes
- 4. Click <OK>
- 5. Click **<OK>** once more

List of available Options	Coption Adjustm	ient	OK
009 EnableDialogAutoHelp 010 EnableAxisShading 011 DezimaDelimiter 012 DefaultMeasurementUnits 013 RoundCalculationMatrix	Description :	Set this option to disable the automatic blank substration mode use for the BC control.	Help
014 DisableAutoBlankSubstra 015 DisableWarpingMessages *	Value ·	Yet	

Next, follow the instructions until step 29 as described in paragraph 8.2.1 for a single endpoint measurement.

If you want to use an already existing protocol you may proceed with the next paragraph.

Options Matrix

For further calculations of the measurements follow the next steps:

- 30. Go to **Options | Matrix** and click **<Add Matrix>**, click **<Add Matrix>** again
- 31. Highlight Matrix No. 3, click **<Edit Matrix>** and rename to e.g. **Blank subtr**

Do the same for Matrix No. 4 (rename to e.g. **Conc**) and Matrix No. 5 (rename to e.g. **Average**)

32. Click <OK>

For export und print you have to refer to this matrices.

- 33. Change the view to the Template section
- 34. Click on the **Partition** tab and select a pattern matching your replicates in the **Plate Partition** drop box
- 35. Click the double-cross to assign for all wells



Matrix Name	Matrix Number	
Sample_ID Reader_Values Blank subtr	Matrix No. 1, (MA1) Matrix No. 2, (MA2) Matrix No. 3, (MA3)	Add Matrix
Conc Average	Matrix No. 4, (MA4) Matrix No. 5, (MA5)	Edit Matrix
		Delete Matrix

MikroMin 2000

🗾 Eile Edit	View	Read	Instrument	<u>Options</u>	Installation	Window	<u>H</u> elp
New	Open	1	Q Read	Print	Export	* Data	⊡ Template
₽ Data		— F	Plate Partition :	Duplicate	s horizontal	•	
- Measure - Sample I	ment Data dentifier	•	# 1		2	3	4
- Error Info - Dilution F - Template - Partition - Groups - Overlay	ormation Factor		A				
- Controls	ls						



- 36. Click on the Controls tab
- 37. Select Blank (BC) in the Control Type drop box
- Click into wells A1/A2 to assign the blank to these wells
- 39. Click **<Yes>** in *Warning! Source matrix*... dialogue
- 40. Select Reader_Values in Source Matrix drop box



- 41. Click on the **Standards** tab
- 42. Click into **Standard Concentration** field and enter the concentration of the first standard
- 43. Select the matching dilution in the **Dilution Fac**tor drop box
- 44. Click into B1/B2 (resp. the set of wells containing the **first standard concentration**) and drag the mouse to the set of wells with the last standard concentration

In case you work with non-regular concentration series, click into the first set of wells, enter the concentration and hit the **ENTER** key

Go ahead until the last concentration is being entered

🛛 <u>F</u> ile Edit <u>V</u> iew <u>R</u> e	ad I <u>n</u> stru	ment <u>O</u> ptions	Installati	ion <u>W</u> in	dow <u>F</u>	lelp		
New Open	Read	Print	Export	Da	ta	Template	Calcul	بيا atio
∃-Data	Standard	Concentration :	1.0000	•	Dilu	tion Factor :	10:1	-
- Measurement Data - Sample Identifier	#	1	2	3		4	5	
- Error Information - Dilution Factor - Template - Partition - Groups - Diverlau	A	Unused			Unused			Un
- Controls - Standards - Calculation - Sample_ID - Reader_Values	в	1.0000			Unused			Un
- Blank subtr - Conc - Average ∋ Result - Sample_ID - Reader Values	с	10.0000			Unused			Un
Blank subtr Conc Average Statistics General Statistics	D	100.0000			Unused			Un
User Statistics 2 User Statistics 3 User Statistics 3 Graphics Bar Graphics Color Graphics	E	1000.0000			Unused			Uni
- Curve Fit - Kinetics - Scanning - Selection - Control History	F	10000.000			Unused			Uni
	G	100000.00			Unused			Uni
	н	1000000.0			Unused			Un
MikroWin 2000	A Partit	ion 👫 Groups		erlav 🖊		s []] Sh	andards	



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Operating Manual

- 45. Click **<Yes>** in *Warning! Source matrix*... dialogue
- 46. Select the Source Matrix, e.g. Blank Subtr
- 47. Select the curve fit Algorithm, e.g. Smoothed Cubic Spline
- 48. Define the \boldsymbol{X} and \boldsymbol{Y} axis scales, e.g. log for both
- 49. Type or select the axes Units
- 50. Click on <Options>
- 51. Select the **Type of Data**, whether they span a linear or a logarithmic range
- 52. Smoothing Factor can be kept as Automatic
- 53. Curve Extrapolation may be checked
- 54. Click **<OK>**
- 55. Click **<OK>** once more
- 56. Change the view to the Calculation section
- 57. Click on the ${f 3}$ Blank subtr tab
- 58. Type MA2 BC into the Calculation Formula: MA2 = matrix 2 = contains the reader values BC = Blank Control
- 59. Click the double-cross to assign for all wells



60. Proceed with the two other matrices alike:

4 Conc

FIT(MA3)

- FIT = curve fitting
- MA3 = matrix 3 = the fit concentration calculation is applied to the values of matrix 3

5 Average AVE(MA4)

AVE = calculation of mean value





MA2 - BC	FIT(MA3)	AVE(MA4)
MA2 - BC	FIT(MA3)	AVE(MA4)
3 Blank subtr 4	ık subtr 🧳 Conc	3 5 Average

MA4 = matrix 2 = calculation is done on the values of matrix 4 (in this case the concentrations)

Should you wish to define any additional calculations please refer to the Mikrowin manual.

- 44. To activate automatic export click **Export Set**up... in the **File** menu
- 45. Select the appropriate and pre-defined export driver

The export drivers and their setup are explained in a later chapter. Please refer to this chapter for the proper configuration of the export driver.

List of Active Export Driver Uits of export driver List of export driver Line Export Driver Help Help Help Help	Deriving Data Rec	kroWin 2000	
List of export driver List of export driver Line Export Driver RawData Export Driver	st of Active Export Driver		OK
List of export driver Line Export Driver RawData Export Driver	List of export driver	-	Cancel
Matrix Export Driver RawData Export Driver	ist of export driver ine Export Driver		Help
Graphic Export Driver	Matrix Export Driver RawData Export Driver Graphic Export Driver		
			F

- 46. Click **<OK>**
- 47. Click Print Setup... in the File menu
- 48. Select the data set by highlighting and clicking <**Add>**

Page Header	header part
File Names	par and dat files names
Measurement Data	raw data
Sample ID	sample info (matrix1)*
Reader_Values	measured data (matrix 2)*
Blank subtr	blank corrected (matrix 3)*
Conc	calc. conc. (matrix 4)*
Average	Averages (matrix 5)*
Gen. Statistics	measurement settings
Curvefit Graphics	standard curve

* the selection and content depends on the matrix definition done in the Calculation section

49. Check if Automatic Print-out is required

50. Type a header and/or footer

Device Date Reduction From its Roots Scriptation Advantation [Header Margins] Available tons Service Data	Print Setup						_
Completion Automation Header Margins Available Rems Provide Rems		Deriving	Data Rec	kroW	in 200	0	
Available toms Solicital tems Pare hance Pape Hance Pare hance Pape Hance Pare hance Pape Hance Pare hance Pape Hance Pape Hance Pape Hance Porter Pape Hance Porter Pape Hance Print Setup Pater OK Cancel Pirter Pape Hance Pater OK Cancel Pirter OK Cancel Pirter OK <td>Compilation Automation</td> <td>Header Man</td> <td>nins</td> <td></td> <td></td> <td></td> <td></td>	Compilation Automation	Header Man	nins				
Provide Name Measurement Data SampleO < Marco > SampleO < Concellent statistics 1 User statistics 1 User statistics 1	Avaiable items			Select	ed items		
Print Setup OK Cancel Pirter Help Print Setup OK Cancel Pirter Help Adomation Adomation Header Margins Adomation Header Margins Print Setup OK Cancel Pirter Help Completion Adomation Header Margins Header Fort : Atal Standard, Spt Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder	Pane Header File Names Measurement Data Sample_ID Reader_Values Results General statistics User statistics 1 User statistics 2 User statistics 3 Bar Graphics		¢	Pag File San Res Ger	e Header Names nple_ID < Ma nder_Values uits < Matrix neral statistic	strix > < Matrix > s	>
OK Cancel Preter Help Print Setup Print Setup Deriving Data Reduction From its Root Completion Automation Header Margins Print Setup OK Cancel Preter Help Print Setup Deriving Data Reduction From its Root Completion Automation Header Margins Header Fort : Anal Standard, 9 pt Set Fort Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder Foder F	Format :	-			Add	Rem	ove
Print Setup Print Setup Print Setup Print Setup OK Cancel Printer Heb Print Setup OK Cancel Printer Heb Print Setup OK Cancel Printer Heb Print Setup OK Cancel Setup Set Fort Header Margins Nationation Header Marg		ок	Ca	ncel	Printer		Help
	Print Setup		-		-	-	
Completion Automation Header Margins Automation P Automatic protout after measurement OK Cancel Printer Help Print Setup Mikro Win 2000 Denning Data Reduction From its Roos Completion Automation Header Margins Header Fort : Avail Standard, Spt		Deriving	Mi Data Rec	kroW	in 200	0	
Advandion Advandion Advandion Advandio	Completion Automation	Hander Mar	nine l				
Print Setup Print Setup MikroWin 2000 Derving Data Reduction From its Roots Completion Adomation Header Margins Header Header Fort : Areal Standard, Bpt Set Fort	Automatic printout	t after measuren	nent				
e Print Setup	P Automatic printout	t after measuren	nent	ncel	Printer		Help
MikroWin 2000 Deriving Data Reduction From its Roots Completion Automation Header Margins Header Header Front : Anal Standard, Spt Footer	✓ Automatic printout	t after measuren	nent	ncel	Printer		Help
Denving Data Holdclob From its Roots Completion Automation Header Margins Pleader Header Font : Arial Standard, Spt Foder Foder Foder	✓ Automatic printoul Print Setup	after measuren	nerit	ncel	Printer		Help
Header Header Header Fort : Arail Standard, Spt Footer	Automatic printou	OK	Ca Mi	ncel	Printer		Help
Header Fort : Anal Standard, 3 pt Set Fort	Automatic printout	OK Derving Header	Ca Mi g Data Rec	ncel	Printer /in 2000	2	Help
Header Fort : Anal Standard, Spt Set Fort	Automatic printod Print Setup Compilation Automation Header	OK Deriving Header Mar	Ca Ca g Data Rec gins	ncel	Printer /in 2000		Heip
	Automatic printout Print Setup Completion Automation Header	OK Deriving Header Mar	nent Ca Data Rec gins	ncel	Printer Jin 2001		Help
	Automatic printout Print Setup Completion Automation Header Header Font : Arail	OK Deriving Header Mar	nent Ca Mi gons	ncel	Pirter Fin 2000	Set F	Help
	Automatic printout Print Setup Compliation Automation Header Header Font : Anal Footer	OK Deriving Header Mar	nent Ca Mi J Data Rec	ncel krow	Pinter /in 2001	Set F	Help
OK Cancel Printer Help	Automatic printout Print Setup Compilation Automation Header Header Font : Anal Footer Footer	OK Deriving Header Mar	Ca Ca Mi j Data Rec gras	ncel	Pirter Fin 2000	Set F	Help



- 52. Go to File | Save As...
- 53. Create and/or Select an appropriate directory, e.g. *ParaTriStar2*
- 54. Select the file type Mikrowin Para File (*.par)
- 55. Type a meaningful **file name**
- 56. Click <Save>

Save in:	🔒 Para		← 🗈 💣 💷 ◄	
C.	Name	*	Date modified	Туре
Recent Places	testtraining.	par	02/28/2011 16:51	PAR File
Network	•	III		
	File name:		•	Save
	Save as type:	MikroWin Para File (* par)	-	Cancel

8.7.2 Measurement with a Curvefit parameter file

The protocol that has been created will be pre-selected. In case you want to perform another measurement you may simply select another protocol from the list.

- Note: In case injectors are to be used for reagent additions make sure the injection lines are properly cleaned and filled (primed). See <u>chapter 9</u> of this manual.
- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.

Note: Make sure the appropriate plate frame is inserted

- 11. Click Open in the File menu
- 12. Select File of type: Mikrowin Para File
- 13. Select the appropriate file
- 14. Click <Open>

Look in	🍶 Para Tri Star 2 💌	🗕 🗈 📩 🖬 🕇	
C.	Name	Date modified	Туре
Recent Places	🖻 singlelumiwithinj.par	06/27/2012 10:59	PAR File
Desktop			
Libraries			
Computer			
Network			
	•		
	File name:	•	Open
	Files of type: MikroWin Para File (* par)	·	Cancel

15. Enter a **file name** under which the measurement is to be stored




Operating Manual

- 16. Click <Start>
- 17. Insert the **microplate** with your samples: well A1 facing the rear and left

Use the **black frame** for microplates with plate heights of 15 mm (\pm 1 mm), e.g. 96 and 384 well plates

Use the **red frame** for microplates with plate heights of 20 mm (\pm 1 mm), e.g. 6, 12, 24 well plates

- 18. Click **<OK>**
- The selected wells of the microplate will be measured and the numerical value of the signal will be displayed

BertholdTech TriStar2	×
Load plate to continue	
ОК	Cancel

0.0

New Open	(Rept	Print	2 Expert	Dete	Template C	Situation B	ante Statut	ies Graphic	100			1	_
∋ Osta	Value o	Poster C06	0	FILU									
- Sample Identifier		1.	2	3	4	5	6	7	8	. 9	10	11	12
Entre Information Dilution Factor Enterplate Pacifion George	A	10	20	10	10	10	٥	0	10	10	٥	10	10
Dvarlag Controls Standards El Colosimon	3	0	0	٥	0	20	10	0	Ð	٥	ø	0	10
Reade_Values Result Differuit Sample_ID Director Values	c	10	10	٥	10	10	- 10	0	0	٥	o	D	D
- Results - Statistics - General Statistics - User Statistics 1	D	0	D	ű.	0	0	0	a	0	0	0	0	(
User Statistics 2 User Statistics 3 Graphics Day Graphics Color Graphics	ε	0	0	0	0	0		0	0	٥	0	0	¢
- Curve FR - Kanatica - Sciencing - Selection - Corecol History	F	0	0	a	0	0	0	0	0	0	Ø	0	t
	8	0	D	0	0	0	0	0	0	0	٥	0	C
-	н	0	0	0	0	0	0	0	D	e	0	0	0
MikroWin 2000 Derving Data Radia Bat	151 Ma	assement Da	. III Sorgie	Identifier	W Englishman	on 10 044	n Factor						
From the Flowler	100	File pane: me	c. today	- 5190	e liniecting		-	Om					

20. Select **Unload Plate** in the **Instrument** menu to retrieve the microplate (still in measurement position) and remove it from the instrument





8.8 Export and print-out in Mikrowin

The export of (calculated) results and raw (measurement) data is carried out via export drivers. The export drivers have to be installed and configured. Data can be exported in XLS and TXT file formats.

8.8.1 Export Driver Configuration

Export drivers have to be installed if you want to export data. In addition, you have to set up the export driver and you have to specify data structure, data matrices as well as header and footer. Data is exported depending on the driver selected and configured in this dialog box. To use another data format, you can select another driver before running a measurement or set up the selected driver new.

In the **Installation Driver** dialog box, select the **Export** tab to view the available drivers. You may choose:

Instalation Driver			X
Reader CurveFit Export Sample	er 🛛		
Description Matrix Ascii Export Driver Extended Ascii Export Driver Line Ascii Export Driver MatrixXLS Export Standard. Matrix XLS Export Kinetics Dri Matrix XLS Export Configurabi	Version 4.03 4.05 4.11 4.06 4.06 4.06	Library ascii5.xdl ascii6.xdl ascii7.xdl xls1.xdl XLS1.XDL XLS1.XDL XLS1.XDL	Add Driver Delete Driver
		Cancel App	Driver Setup

Selection of export drivers

Matrix Export Driver Driver (template) for export of calculated data with matrix (i.e. plate lay-out) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

Line Export Driver Driver (template) for export of calculated data with list (i.e. tabletype) structure. Only data that are visible on result matrices can be exported. File formats may be EXCEL, Text (ASCII) and CSV.

RawData Export DriverDriver (template) for export of all raw data. File formats may be EXCEL, Text (ASCII) and CSV. Whether data in the export file are presented in list or matrix format depends on the settings and data origin.



<u>Matrix Export Driver</u> If you select the Matrix export driver, you have to define the following configuration:

Matrix Expo	rt Driver, Version	4.11	×
Export Layo	ut		
Header :			Add
Matrix :	Matrix Matrix No 1 Matrix No 2 Matrix No 2 Matrix No 4	Selection mode Matrix identified by Number Matrix identified by Number Matrix identified by Number Matrix identified by Number	Add Edit Delete
Footer :			Add
Operation M	ode		
Export :	always	•	
Add Gener	al Statistics : Yes	•	
Export Targe	et		
Format :	XLS File	Adjustments	OK
Directory :	C:\Programme\Mikro\	Vin 2000\Transfer	Cancel Help

Matrix export driver setup

Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).

Matrix Name	#M×
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI

Context menu for entering header placeholders

Example:

Header with date, time and plate identification, separated by tab characters: **#DT#TB#TM#TB#PI**

Matrix In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. *Make sure* that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.

Click <Add> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list.

Click < Delete> to delete the selected matrix from the matrix list.



Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

ExportThe proper setting is **Always**.

Add General Statistics Options are Yes or No.

Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from Text File, XLS File, CSV File, CommPort and Clipboard.

Target Define the directory which the file is to be exported to. You may use the browse <...> button to locate an appropriate directory.

Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

<u>Line Export Driver</u> Select this export driver to define a table-type file. Parameters (header, matrix and footer as well as the target directory for data storage) are entered in the same manner as for an matrix-type file (see previous section).

Line Export Driver, Version 4.18	\mathbf{X}
Export Layout	
Header :	Add
Line : #01#TB#02#TB#03#TB#04	#TB#05#TB Add
Footer :	Add
Data Selection	Operation Mode
Consider Plate Partition	Export : always
Skip Control Positions	Data Sequence : A, B, C, D, 💌
Skip empty Positions of Matrix 1	Add General Statistics : Yes 💌
Export Target	
Format : XLS File	Adjustments OK
Directory : C:\Programme\MikroWin 20	D0\Transfer Eancel Help

Line export driver setup



Export Layout Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).

Matrix Name	#M×
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI

Context menu for entering header placeholders

Line In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. *Make sure* that the matrices and their numbers specified here is identical with the number of the result matrix in the parameter file.

Date	#DT
Time	#TM
Position (A01)	#PS
Position (A1)	#PO
Error	#ER
Plate Identifier	#PI
Template Identifier	#TI
Test Name	#TS
Tabulator	#TB
Matrix 1	#01
Matrix 2	#02
Matrix 3	#03
Matrix 4	#04
Matrix 5	#05
Matrix 6	#06
Matrix 7	#07
Matrix 8	#08
Matrix 9	#09
Matrix Name	#/Name>

Context menu Line Export Driver

Click <Add> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list. Click <Delete> to delete the selected matrix from the matrix list.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.



Data Selection Define additional options regarding data sources and positioning.

Consider Plate Partition This option should be checked when replicates are used and they are to be exported next to each other.

Skip Control Positions This option may be checked if values of Controls are not supposed to be exported.

Skip Positions without Sample ID Check if only samples with sample IDs are to be exported.

Skip Empty Positions of Matrix 1 This option may be used if the values of unused wells are not to be exported. Matrix 1 must contain an appropriate variable like **MEA** or **LB** 1.

Operation Mode Define additional options.

ExportThe proper setting is **Always**.

Add General Statistics Options are Yes or No.

Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from Text File, XLS File, CSV File, CommPort and Clipboard.

Target Define the directory which the file is to be exported to. You may use the browse <...> button to locate an appropriate directory.

Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

RawData Export Driver With this export driver a file containing all raw data will be created.

For the export of kinetic data the kinetic layout can be selected (see below).

When the Rawdata Export driver is used for values derived from scanning operations each well is displayed in a separate area with the individual reading points displayed in an X-Y matrix representing the scanning positions.

With data coming from multilabel measurements (e.g. BRET) with a single reading per wavelength the data are exported in a respective amount of matrices representing the plate layout.

Data from multi-plate readings (Batch mode) are also exported in a matrix orientation. *Note:* only single readings are supported!



RawData Ex	port Driver, Version 4.16
Export Layo	ut
Header :	Add
RawData	Kinetic Layout : Time / Position 💌 Time Format : hh:mm:ss 💌
Footer :	Add
Operation M Add Gener	lode al Statistics : no ▼ mpleID Information
Export Targ Format : Directory :	et Text File Adjustments C:\Programme\MikroWin 2000\Transfer Help

Export Layout

Define the file layout.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).

Matrix Name	#M×
Date	#DT
Time	#TM
Plate Identifier	#PI
Template Identifier	#TI

Context menu for entering header placeholders

RawData Kinetik Layout

The selection **Position/Time** has a column adressed to each well position (left to right) and the consecutive readings are entered in lines (down).

The selection *Time/Position* has a line adressed to each well position (down) and the consecutive readings are entered in columns (left to right).

Note: Keep in mind that EXCEL supports a maximum of 256 columns.

Time Output Format

Select the time format a kinetic reading. Choices are: *hh:mm:ss* or *sec.msec*.

Footer Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma,



space, etc.). This context menu includes the same options as the header context menu.

Operation Mode Define additional options.

Add General Statistics Options are Yes or No.

Add Sample ID information Check if you want that information added to each value.

Export Target Define the target directory for the file as well as its format. In addition you may have the export file automatically opened.

Format You may select from Text File, XLS File, CSV File, CommPort and Clipboard.

Target Define the directory which the file is to be exported to. You may use the browse <...> button to locate an appropriate directory.

Adjustment You may define an executable command line which is executed after the export, e.g. to open the exported file.

8.8.2 Adding additional / replicating export drivers

Especially in multi-user environment the individual users will have their own demands for export driver setups. To support this multiple copies of the export driver can be installed and each of the copies can be individually set up.

It is recommended for convenience and security to create a new directory within the *Mikrowin 2000* directory (e.g. called "**Drivers**") and copy the original export drivers *matrix1.xdl*, *line1.xdl* and *rawdata1.xdl* to it.

First, rename the export driver that is to be installed a second time by highlighting it in the **Installation | Driver | Export** menu. Hit the **ALT** and the **R** keys simultaneously. You can enter a new name for the driver. Confirm with <**OK**>.

Driver Rename Dialog	
Please enter the new driver name in the edit option below.	ОК
new name	Cancel

Now you can re-install the driver again by the clicking **<Add Driver>** and browsing to the driver directory you created.





Select the respective driver in the dialogue displayed.

Instal	llation Driver Add			×
List	of available drivers			1
0	Description	Version	Library	
Li	ine Export Driver	4.15	line1.xdl	
M	fatrix Export Driver	4.09	matrix1.xdl	
B	lawData Export Driver	4.10	rawdata1.xdl	
			. 1	
	OK	Cano	el	

You may repeat this procedure as often as necessary to get an appropriate number of export drivers.

8.8.3 Automatic export

Choose the menu item **File | Export Setup** to select the export driver that is to be loaded <u>automatically</u> upon successful completion of a reader run. If a driver has been selected for the active parameter file, data evaluation is performed after completion of the respective measurement and data export is carried out in accordance with the selected driver.

Please keep in mind:

This function is only valid for the active parameter file.

Prerequisite for automatic data export is that the respective export driver has been installed and set up in the menu item **Installation | Driver** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**) and the export driver has been selected in the menu item **File | Export** (see chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**).

Open parameter file.

Select File | Export Setup to open the File Export Setup dialog box.



File Export Setup dialog box



List of Active Export Driver

Select the export driver you want to use for automatic data export upon successful completion of a reader run. Click on the arrow button to open the list showing the available drivers and select the driver you want. The selected drivers appear in the text box directly below the drop-down list box.

To delete a driver from the list, select this driver and then click <Remove>.

Click **<OK>** to accept your selection.

You <u>must</u> save the parameter file <u>after</u> the export setup !

8.8.4 Export on demand

The following dialog supports manual export of program data. The data to be exported, the format as well as the export destination depend on the selected driver and its configuration. The actual data export is carried out by an export driver if you click on the **<Export**> button after a measurement.

Open the parameter file you need.

Select File | Export to open the File Export dialog box.

File Export	
Active Export Driver	ОК
Matrix Ascii Export Driver	Cancel
	Help
Export Target Information File Name : Untitled.TXT Directory : C:\Program Files\MikroWin 2000\Tran	Browse

File Export dialog box with open driver list

Active Export Driver Select the export driver you want to use for data export. Click on the arrow button to open the list showing the available drivers and select the driver you want. <u>Please keep in mind</u> that you have set up the driver you have selected here in the menu Installation | Driver | Export. Otherwise, no data will be transferred!

Export Target Information File Name

Shows the file name of the active parameter file. An extension identifying the selected driver is appended (XLS for Excel files and TXT for ASCII files). The file name can be edited.

Directory

The target directory has been defined by the selected export driver during installation. Click the **<Browse**> button to select another target directory.

Click **<OK**> to accept your selection.



The export will be executed automatically if selected in the respective protocol file.



8.8.5 Data Print-out

Data and results can be printed automatically whenever a measurement with a parameter file has been done - *Automatic Print-out* has to be checked as described in the previous chapters – or on demand for the currently loaded data file.

- 1. Go to Print Setup... in the File menu
- 2. Select the appropriate items by highlighting and clicking **<Add>**



- 3. You may check the settings and layout by selecting **Print Preview** in the **File** menu to get a preview of the print-out
- 4. Select **Print** in the **File** menu to start printing the data

The print-out will be executed automatically if checked when created in the respective parameter file.

8.8.6 Print-out of parameter file settings

All settings including the calculations can be printed by clicking **Template Print-out** in the **File** menu. The information will be presented as a HTML file in the web browser from where you can print the content.



9. Maintenance

9.1 Cleaning the Instrument

9.1.1 Cleaning the instrument surface

The **surface** of the instrument is protected by a washable finish. Dirty or dusty surfaces should be cleaned using a damp cloth or optical grade tissue. If necessary, use a mild detergent or diluted EtOH.

Do not use a scouring agent!

9.1.2 Cleaning the inside of the instrument

The inside of the instrument does not need to be cleaned regularly. Only in case liquid spillage it may be necessary to clean the inside.

<u>Do not open the instrument by yourself! Call a Berthold Technologies technical</u> <u>support person.</u>

Before opening the instrument, turn it off and disconnect it from power supply!

Open the screws on the instrument cover to clean the instrument inside. Then detach the cover by moving or lifting it carefully.

Always keep the sample holders and the entire inside of the instrument clean. Wipe off any dirt using a damp cloth or optical grade tissue. Use cotton buds for corners. Remove dirt quickly so it does not get fry and may not have any adverse effect on moving parts.

9.2 Cleaning Tubing

Injector tubing have to be washed

- before starting work
- □ before changing reagents
- □ at the end of each work session before turning off the instrument
- □ after longer periods of inactivity

Use solutions recommended by the kit manufacturer.

Other recommended cleaning reagents are

- o deionised water
- o diluted alcohol: 70 % Ethanol, Propanol
- o 2 5 % hypochlorite solution ("bleach")
- o 0.5 1 M Chloric acid (HCl)
- o 0.5 1 M Sodium hydroxide (NaOH)
- o 0.1 % SDS



Non-foaming detergent (up to 10 %)

Some of these reagents may be hazardous. Please refer to the respective safety instructions (e.g. R and S codes) of the supplier.

Berthold Technologies' cleaning solution CLEANIT Standard (product code 45218) is an efficient and proven cleaning solution for most of the common reagents in use. It is recommended to use this solution at least once a weak to ensure a long lifetime of the injectors!

Injector tubing have to be primed

D prior to each measurement using the respective reagents.

1. Click Injector Settings in the Instrument menu

0

- 2. Define the **default number of wash cycles** 50 is recommended
- 3. check the **use of the Berthold Technologies Washplate** (when available)

Injector Settings		x
Injector Maintenance		
Select Cycles and Injector	S.	
Press Close to Exit.		
Number of Wash cycles:	50	
Perform with Berthold W	/ashplate f Prime cycl	les: [12
_ Injectors		
Injector 1	🔽 Injector 2	Injector 3
Car	ncel Clos	e

4. Click Wash in the Instrument menu

File View Ir	istrument 🔳	ools Help						
	Properties							
Default	Load Plate Unload Plate			Start Measurement				
	Injector Se	ttings		2	4	5	c	
Protocol Des	Wash					-		
	Refresh Unload Injector	ample	Sample	Sample	Sample	Sample		
			_					
R	Excitation Emission F	Filter Slide ilter Slide	ample	Sample	Sample	Sample	Sample	
ReRun & Ec	Boot Instrument	ample	Sample	Sample	Sample	Sample		
	Shipping Brace							
	D	Sample	Sample	Sample	Sample	Sample	Sample	



5. Define the number of **Wash Cycles**

Make sure the total Wash volume does not exceed the volume of the plate being used!

- 6. Select the respective injector(s)
- 7. Click <Next>

- 8. Load a Wash plate
- 9. Click <Next>

10.	Attach the reserv	voir containir	ng the a	ppropriate-
	Wash Solution	(see above)		

11. Click **<Next>**

jector Sequence		X
Injector Wash Sequenc	e	
Select Injectors.		
When finished, press N	ext.	
Number of Wash cycle	s: 🔟	
Perform with Berthole	d Washplate	
200		

ector Sequence		
Injector Wash Sequence	9	
Load empty Wash Plate		
Number of Wash cycle:	s: 50	
Perform with Berthold	Washplate	
IN LOW ON LOW LAND		

njector Sequence		×
Injector Wash Sequenc	9	
Load Wash Solution in t	he Reagent Positions Sele	ected.
When finished, press N	ext.	
Number of Wash cycle	s: 50	
Perform with Berthold	l Washplate	
_ Injectors		
Injector 1	🔽 Injector 2	🔽 Injector 3
-	1	
(Cancel Nex	d >>



13. Click <Close>

12. Wait until the wash cycles are completed

njector Sequence		×
Injector Wash Sequence	9	
Injecting Wash Solution.	2	
Please wait for cycle to	complete.	
Number of Wash cycles	50	
Perform with Berthold	Washplate	
_ Injectors		
Injector 1	🔽 Injector 2	Injector 3
C	ancel	d>>>

injector Sequence		×
Injector Wash Sequence	e	
Injector Wash Sequence	e Finished.	
Please remove plate pr	ior to running a new measu	irement.
Press Close to Exit.		
Number of Wash cycle:	s: 1 IWashplate	
_ Injectors		
🔽 Injector 1	🔽 Injector 2	🔽 Injector 3
	Cancel	ISE

14. **Remove Wash plate** by clicking **Unload Plate** in the Instrument menu

File View	Instrument T	ools Help			
🗅 😂 🖬 🌡	Properties		-		
Default	Load Plate Unload Plat	te		🕨 Start Mea	isurement
	Injector Set Prime Wash	ttings	2	3	4
Protocol Des	Refresh	retor	Sample	Sample	Sample
6	Excitation F Emission Fi Plate Editor	Filter Slide ilter Slide r	Sample	Sample	Sample
ReRun & Ec	Boot Instru	Instrument Sampl		Sample	Sample

Note: It is recommended to leave deionised water in the injection lines during idle periods of hours up to a few days.



Only in case the instrument stays idle for multiple days up to weeks it is recommended to empty the lines by starting the Wash procedure without a wash solution.

9.3 Priming Tubing

9.3.1 Priming before Measurement

Injection lines have to be primed (filled) prior to measurements which require the use of injectors for reagent addition.

Note: It is strongly recommended to perform the priming with deionized water first and leaving the lines filled with deionized water before priming with reagents.

This procedure avoids reagents aerosol splashes at the injector tips and thus contamination of the instrument.

- Note: Do not overfill the reagent trough as liquid spills in the injector compartment may cause severe damage to the electrical system. Take special care when ice in the trough starts to melt.
- 1. Click Injector Settings in the Instrument menu
- Define the default number of prime cycles 12 is recommended – which will be used for the default priming and check the use of the Berthold Technologies Washplate

ijector Settings			×
Injector Maintenance			
Select Cycles and Injec	tors.		
Press Close to Exit.			
Number of Wash cycle	s: 50		
Perform with Berthold	l Washplate	f Prime cycles	12
_ Injectors]
Injector 1	🔽 Inje	ector 2	Injector 3
	Cancel	Close	



3. Click Prime in the Instrument menu

Instrument Con	trol and Eval	luation					The State
File View Ir	istrument	Tools Help					
i 🗅 💕 🖬 d	Propertie	es					
Default	Load Pla Unload P	te Plate		Start Measurement			
	Injector S	Settings					
	Prime			3	4	5	6
	Wash		-				
Protocol Des	Refresh	ample	Sample	Sample	Sample	Sample	
	Unload I	Unload Injector		-			
R.	Excitation Emission	n Filter Slide 1 Filter Slide	ample	Sample	Sample	Sample	Sample
ReRun & Ec	Boot Inst	tor	ample	Sample	Sample	Sample	Sample
	Shipping	Shipping Brace		_		_	
	D	Sample	Sample	Sample	Sample	Sample	Sample
		-					

- 4. Check Berthold **Washplate** (when available)
- 5. Select the prime mode
 - a. Check **Use Customized Prime** to select a user defined method (see <u>next chapter</u>)
 - b. Uncheck Use Customized Prime to use the default prime mode
- 6. Select the respective injector(s)
- 7. Click <Next>

njector Sequence		X
Injector Prime Sequence		
Select Injectors.		
When finished, press Nex	L	
Perform with Berthold V	/ashplate	
🔽 Use Customized Prime	100default_01	•
_ Injectors		
🔽 Injector 1	🔽 Injector 2	Injector 3
Ca	ncel Next	·>>

- 8. Load a prime plate / wash plate
- 9. Click <Next>

njector Sequence		×
Injector Prime Sequence	2	
Load empty Prime Plate		
Perform with Berthold	Washplate	
🔽 Use Customized Prim	ne 100default_01	-
_ Injectors		
🔽 Injector 1	Injector 2	Injector 3
C	ancel Next	>>



Operating Manual

- 10. Attach the reservoir(s) containing the appropriate **Assay Reagents** (or deionized water; see above)
- 11. Click <Next>

viector Prime Sequence	
oad Reagent in the Reag	ent Positions Selected.
/hen finished, press Next.	
Perform with Berthold W	ashplate
⁷ Perform with Berthold W ⁷ Use Customized Prime	ashplate
 Perform with Berthold W Use Customized Prime 	ashplate
Perform with Berthold W Use Customized Prime Injectors	ashplate 100default_01
Perform with Berthold W Use Customized Prime Injectors Injector 1	ashplate 100default_01
Perform with Berthold W Use Customized Prime Injectors Injector 1	ashplate 100default_01

12. Wait for the Prime procedure to be finished for one injector

Injector Prime Sequence		
Injecting Reagent.		
Please wait for cycle to comp	olete.	
Perform with Berthold Was	shplate	
🔽 Use Customized Prime	100default_01	•
la la ataux		
-Injectors	E Injector 2	E Injector 3
	i injector c	1 Injector 5
I♥ IIIJector I		

13. Click <Close>

Injector Prime Sequence Injector Prime Sequence Finished. Please remove plate prior to running a new measurement. Press Close to Exit. I Perform with Berthold Washplate Use Customized Prime Injectors Injectors Injector 1 Injector 2 Injector 3		
Injector Prime Sequence Finished. Please remove plate prior to running a new measurement. Press Close to Exit. Press Close to Exit. Perform with Berthold Washplate Use Customized Prime 100default_01 Injectors Injector 1 Injector 2 Injector 3	Injector Prime Sequence	9
Please remove plate prior to running a new measurement. Press Close to Exit. Preform with Berthold Washplate	Injector Prime Sequence	e Finished.
Press Close to Exit. Perform with Berthold Washplate Use Customized Prime Injectors Injector 1 Injector 2 Injector 3	Please remove plate pr	ior to running a new measurement.
Perform with Berthold Washplate Use Customized Prime 100default_01 Injectors Injector 1 Injector 2 Injector 3	Press Close to Exit.	
Image: Vision of the second secon		
_ Injectors ↓ Injector 1 □ Injector 2 □ Injector 3	Perform with Berthold	l Washplate
I Injector 1 I Injector 2 I Injector 3	 Perform with Berthold Use Customized Print 	IWashplate 1e 100default_01 _
	Perform with Bertholc Use Customized Prin Injectors	IWashplate ne 100default_01 _



Operating Manual

14. **Remove prime / wash plate** by clicking **Unload Plate** in the Instrument menu

File View	Instrument	Tools Help			
🗅 😂 🖬 👌	Propert	ies	-		
Default	Load Pl Unload	ate Plate		🕨 Start Mea	isurement
	Injector Prime Wash	Settings	2	3	4
Protocol Des	Refresh		Sample	Sample	Sample
6	Excitation Emission Plate Eco	on Filter Slide on Filter Slide ditor	Sample	Sample	Sample
ReRun & Ec	Boot In	strument	Sample	Sample	Sample

15. The instrument is now ready for use



9.3.2 Customizing the Priming Sequence

Some reagents (e.g. high viscosity) or solutions (e.g. cells) require special priming procedures which can be defined individually.

1. Click **Prime Customize** in the **Tools** menu



2. The Prime Customize dialog will be displayed

ime Customize		
Operations	Sequence	
B- ▲ Instant B- ◯ Delay B- S Oscillate B- △ Ventilate B- △ Prime		
	beoj -	
	Enter File Name	

clicking on \boxdot in the Sequence window will expand the respective folders and display the settings



3. The respective operation can be selected for the prime sequence by dragging it from the left column to the right column (**Sequence**)



to change the sequence the arrow buttons can be used



to remove an operation the button 🙆 can be used

Ť
-
*

The operations and their settings:

To change the settings

- expand the operation
- expand the setting
- click onto the number
- click onto the number a 2nd time
- type the appropriate number
- confirm with the ENTER key
- a. Injection

the injector is filled with the max. injection volume from the reagent reservoir and injects the set volume

Volume	percentage of the max. inj. vol.
Delay	delay before the operation in sec
Speed	1 10
Repeats	number of repeats

b. Delay

a delay time that elapses between operations, e.g. to mimic the injection timing of the assay (this can be important with a cell suspension)

Delay elapsing time in sec

c. Oscillate

the injector is (partly) filled and oscillates between the set positions (back into the reagent reservoir)

ol.
)

End Point percentage of the max. inj. vol.

Speed 1 ... 10

Repeats number of repeats

d. Ventilate

the injector is completely filled (beyond the max. injection volume) from the reagent reservoir and injects the total volume of the bellow



01

Operations	
🗆 🖕 Volume [%]:	
Delay [s]:	
0.1	
Speed:	
B- A Repeats:	
1 - L A	









Delay	delay before the operation in sec
Speed	1 10
Repeats	number of repeats

e. Prime

the injector is filled with the max. injection volume from the reagent reservoir and injects the full volume

Delay	delay before the operation in sec
Speed	1 10
Repeats	number of repeats



4. After the sequence is completed enter a **name** for this priming sequence and click **<Save>**

the file will get the extension .wpe

the respective directory will be displayed

my_prime_1	S
C:\LIsers\Hutter\Documents\ICE	Paral umat3\mv_prime

	×
	Close

5. Close the dialog by clicking

- 1 0		_ injection	volume [x]:	100	Delay [s]:	0.1	Speed: 10	Repeats: 1	
Delay		Injection	volume [8]:	10	Delay [s]:	0.1	Speed: 10	Repeats: 10	
E O Delay [s]:		- 🕑 Delay			Delay [s]:	1			
01		Injection	I[H] smulov	50	pelay [s]:	0.1	Speed: 10	Repeats: 1	
12 Oscillate		Opelay			Delay [s]:	1			×
B Sating Point [1]		-S Oscillate	Starting Point	[9]: 20	End Point [%]:	70	Speed: 10	Repeats: 10	(m)
-== 20	63	O Prime			Delay [s]:	0.1	Speed: 10	Repeats: 5	
- Sa and Port [1]:									
									X Clearist
0									
E Sa Papears.									
o vertilite	1								
o mme									
E O Const Int									
0 0.1									
E Speed									
0 10									
E 🛆 Repeats									
0 0	1.00								
	-								
			-	C. Lord					
			1. The second	Food					



9.4 Emptying Tubing

This operation can be used to empty the injection lines after the measurement and re-collect valuable reagents in the reagent reservoirs.

Note: Make sure the reagent reservoir are connected to the injection tubings !

1. Click Unload Injector in the Instrument menu



2. Define the Number of Unload cycles

each cycle is equivalent to the max. injection volume of the injector installed

a minimum of 10 is recommended

- 3. Define whether **Injector 1** or **Injector 2** or **Injector 3** or any combination are to be emptied
- 4. Click <Next>

Injector Sequence
Injector Unload Sequence
Select Injectors.
When finished, press Next.
Number of Unload cycles:
Perform with Berthold Washplate
Injectors
Injectors
Injector 1
Injector 2
Injector 3
Cancel
Next >>

5. Wait for the unload operation to be finished

njector Unload Sequer	ice	
njector Unload Sequer	nce Finished.	
Press Close to Exit.		
Number of Unload cyc	les: 1	
Perform with Berthol	d Washplate	
-Injectors		
	E 1 1 1 0	Injector 3



6. Click **<Close>**

jector Unioad Sequen	Ce	
jector Unload Sequen	ce Finished.	
Press Close to Exit.		
Number of Unload cycl	es: 1	
Perform with Berthold	Washplate	
Injectors		
IIIELUIS		



9.5 Preparations for transport

The following safety provisions have to be taken to transport or ship the instrument:

- Turn instrument off and **disconnect it from mains**
- Insert transport safety device(s)



- For shipping you **must** use the **original transportation case**
- Encase the instrument with the styrofoam parts
- Tape shipping carton tightly
- Have a filled in **Declaration on Decontamination** accompany the instrument when shipping back to Berthold Technologies or one of its representatives



10. Trouble shooting

Symptom	Possible cause	Solution
LED flashes red accompa- nied by 2 beeps	CAN module not correctly in- stalled	 switch instrument off and on again
		2) call service
LED stays orange	Cable between instrument and computer is not connected	1) attach cable properly
	Wrong COM assigned	 use service software and run "Scan COM ports" command
Instrument does not respond to software commands (sta-	Cable between instrument and computer is not connected	1) attach cable properly
tus Timeout Error)	Wrong COM assigned	 use service software and run "Scan COM ports" command
LED stays dark	Instrument not switched on	1) switch instrument on
	Mains not plugged in	2) plug in mains
	mains supply deactivated	 check with local house electrician
	mains plug defective	4) call service
Lower signal than expected	Pipetting/preparation error	 verify by checking replicate and other samples / con- trols / standards and prepare faulty sample again
	substrate consumed	 prepare new plate and read immediately after add- ing substrate
Signal not above back-	No sample	1) check sample preparation
ground readings	No reagents added	2) add reagents
No signal at all	Faulty PMT	Call service
Plate is not moved to meas- urement position	Plate not correctly inserted	1) insert plate correctly
	Wrong frame	2) change frame
	Plate too high	 use another plate with a to- tal max. height of 16 or 21 mm respectively



Error message no plate	No plate	1) insert plate
	Wrong frame	 insert black frame for 15 mm plates
High background signal	Reagents not prepared properly	3) prepare reagents properly
	Reagents contaminated	4) prepare fresh reagents
	Plate contaminated	5) use another clean plate
		6) call service
Standard curve cannot be calculated	Standards pipetted in wrong or- der	 prepare new plate with cor- rect layout of standards
		 use the edit function in the standard curve tab
Excel Files cannot be opened	Excel is not installed	Install Excel
Adobe PDF files cannot be opened	Adobe Acrobat Reader is not in- stalled	Install Adobe Acrobat



11. Technical Data

Mains Supply	100 – 240 VAC			
	Fluctuations must not exceed ± 10 %; max. voltage 253 VAC			
	50 / 60 Hz			
Operating voltage	24 VDC			
Power consumption	220 VA			
Certifications	CE, UL, CSA			
Safety standards	EN 61010-1, EN 61326-1, EN 61000-3-2, EN 61000-3-3			
Installation category	П			
Temperature range	storage: 0° - 40°C			
	operation: 15° - 35°C			
Humidity	10 – 80%, not condensing			
	maximum relative humidity of 80 % for temperatures up to 31 °C decreas-			
	ing linearly to 50 % relative humidity up to 40 °C			
Altitude	< 2000 m (above sea level)			
Pollution degree	2			
Dimensions	391 x 470 x 344 mm (W x D x H)			
Weight	21 kg			
Plate formats	6 to 384 well, solid and strip			
	Dimensions 128 x 86 mm (L x W), height 14.0 – 21.0 mm (adapters nec-			
	essary			
	Petri dishes 35 and 60 mm			
Measurement tech-				
nology	Fluorescence			
	Absorbance			
Operation modes	Integral measurement: 0.1 – 200 s			
	Dual integral measurement: 0.1 – 200 s each			
	Kinetic			
	Repeated			
	Scanning			
	Delay: 0 – 600 s			
	Shaking			
	Injection			
Detector	Photomultiplier operated in single photon counting technology			
Sensitivity	Luminescence:			
	ATP: 6 amol/well (96)			
	Fluorescence:			
	FTIC: 0.3 tmol/well (384)			
	Absorbance:			
	Accuracy better 2 %, precision better 0.6 % (96 well, 2 OD)			
Dynamic Range	6 orders of magnitude			
Crosstalk	10 ⁻⁶ (black plates)			
Interface(s)	USB			
Operating system	Win Vista, Win 7			



PC requirements	Pentium, 500 MHz (or better), CD ROM drive, USB port			
Software	wizard support for parameter entries			
	input of plate format			
	selection of wells			
	raw data assays (reporter genes, caspases, etc)			
	dual raw data assays (e.g. dual reporter genes)			
	kinetic			
	repeated			
	scanning			
	ratio calculation or subtraction			
	data export: EXCEL			



12. Appendix

a. Customer Reply Form

Send Customer Reply Form to:

Berthold Technologies GmbH & Co KG Technical Support Calmbacher Str. 22 75323 Bad Wildbad Germany Phone: +49 7081 177 114 Fax: +49 7081 177 301 Email: service@berthold.com

or your local representative.

A blank Customer Reply Form can be found overleaf.



BERTHOLD TECHNOLOGIES

Customer Reply Form	
Date: 0	Customer no.:
Name:	
Company:	
Department:	
Address:	
Address:	
Phone:	Fax:
Email:	
Instrument:	
ID no.:	
Serial no.:	
Embedded software version:	
Instrument driver software version:	
Accessory instruments:	
PC Software:	PC software version:
Windows version:	
Computer type:	CPU type:
Other installed software:	
Time when problem occurred (Windo	ws clock):
Error message(s):	
Description of the problem:	

b. Confirmation on Decontamination Form

Confirmation on Decontamination

If you return an instrument to BERTHOLD TECHNOLOGIES for servicing purposes which is not properly decontaminated, there will be a health risk for BERTHOLD TECHNOLOGIES employees. We therefore need your confirmation that the instrument was decontaminated and cleaned properly before shipping. If the form below is not filled in accordingly and completely, we are forced to reject the instrument. Please understand that this is intended to protect our employees from any hazards.

Please put one copy into the shipping box and a duplicate into an envelope attached to the outside.

	(Please use capit	
instru	ment / component:	serial no.:
instru	iment or component has come into conta	act with:
[]	radioactive substances	means of decontamination applied:
	Isotope(s):	
[]	chemical reagents	means of decontamination applied:
	specify:	
	R and S rules:	
[]	biological material	means of decontamination applied:
	specifiy:	
[]	contagious agents	means of decontamination applied:
	specify:	
	indicate security level of the laboratory	the instrument has been used in
	[]S1 []S2 []S	53 []S4
[]	I hereby confirm that the instrument or contaminated with any of the above me	component specified above was not entioned substances / reagents / agents
[]	I hereby confirm that the instrument or o	component specified above was
	decontaminated / cleansed using the ap	ppropriate method
date:		signature:
name	»:	address:
title:		
phon	e:	
fax:		7-40.12 – 31531/03



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