labtech.com

Instructions for Use for
Absorbance Reader

LT-4500

Document Name: LT-4500 IFU
Document Revision No.: 1.0

CE

WARNING

CAREFULLY READ AND FOLLOW THE INSTRUCTIONS FOR USE BEFORE OPERATING THE INSTRUMENT.

Notice

Every effort has been made to avoid errors in text and diagrams; however, labtech.com assumes no responsibility for any errors, which may appear in this document.

It is the policy of **labtech.com** to improve products as new techniques and components become available. Therefore, **labtech.com** reserves the right to change specifications at any time *with appropriate verification, validation, and approvals.*

We would appreciate any comments on this document.

Manufacturer

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Copyright Information

About the Instructions for Use

Original instructions. This document is intended as ***Instructions for Use*** (IFU) for the **labtech.com** LT-4500 absorbance reader, which is designed to measure the light absorbance (optical density) of samples in 96-well microplates. It is intended as reference and instruction for the user.

This document instructs how to:

- Install the instrument
- Operate the instrument
- Clean and maintain the instrument

Remarks on Screenshots

The version number displayed in screenshots may not always be the one of the currently released version. Screenshots are replaced only if the content related to application has changed.

Warnings, Cautions, and Notes

The following types of notices are used throughout this publication to highlight important information or to warn the user of potentially dangerous situations:

Note
Gives helpful information.

CAUTION
INDICATES A POSSIBILITY OF INSTRUMENT DAMAGE OR DATA LOSS IF INSTRUCTIONS ARE NOT FOLLOWED.

WARNING
INDICATES THE POSSIBILITY OF SEVERE PERSONAL INJURY, LOSS OF LIFE OR EQUIPMENT DAMAGE IF THE INSTRUCTIONS ARE NOT FOLLOWED.

WARNING
INDICATES THE POSSIBLE PRESENCE OF BIOLOGICALLY HAZARDOUS MATERIAL. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

WARNING
THIS SYMBOL INDICATES THE POSSIBLE PRESENCE OF FLAMMABLE MATERIALS AND A RISK OF FIRE. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

ATTENTION

DIRECTIVE 2002/96/EC ON WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE)

NEGATIVE ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE TREATMENT OF WASTE.

- **DO NOT TREAT ELECTRICAL AND ELECTRONIC EQUIPMENT AS UNSORTED MUNICIPAL WASTE.**
- **COLLECT WASTE FROM ELECTRICAL AND ELECTRONIC EQUIPMENT SEPARATELY.**

Table of Contents

1. Safety

1.1 Instrument Safety

1. Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
2. Read and understand all information in the Instructions for Use (IFU). Failure to read, understand, and follow the instructions in this document may result in damage to the product, injury to operating personnel, or poor instrument performance. **labtech.com** is not responsible for damage or injuries resulting from incorrect handling of the device.
3. Observe all WARNING and CAUTION statements in this document.
4. Always disconnect the device from the main power supply prior to cleaning and disinfection.
5. Never open the instrument's housing.
6. Observe proper laboratory safety precautions such as wearing protective clothing (e.g. gloves, lab coat, and safety glasses) and the application of approved laboratory safety procedures.

CAUTION
IF THE INSTRUCTIONS GIVEN IN THIS PUBLICATION ARE NOT CORRECTLY FOLLOWED, THE INSTRUMENT MAY BECOME DAMAGED OR PROCEDURES MAY NOT BE CORRECTLY PERFORMED AND THE SAFETY OF THE INSTRUMENT CANNOT BE GUARANTEED.

It is assumed that the instrument operators, because of their vocational experience, are familiar with the necessary safety precautions for handling chemicals and bio-hazardous substances.

Adhere to the following laws and guidelines:

- National industrial protection law
- Accident prevention regulations
- Safety data sheets of the reagent manufacturers

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE LT-4500 MAY COME IN CONTACT WITH BIOHAZARDOUS/INFECTIOUS MATERIAL.

MAKE SURE THAT ONLY QUALIFIED PERSONNEL OPERATE THE INSTRUMENT. IN CASE OF SERVICE OR WHEN RELOCATING OR DISPOSING OF THE INSTRUMENT, ALWAYS DISINFECT THE INSTRUMENT ACCORDING TO THE INSTRUCTIONS GIVEN IN THIS DOCUMENT.

OBSERVE PROPER LABORATORY SAFETY PRECAUTIONS SUCH AS WEARING PROTECTIVE CLOTHING WHEN WORKING WITH POTENTIALLY INFECTIOUS SUBSTANCES.

WARNING

THE INSTRUMENT COMPLIES WITH THE EMISSION AND IMMUNITY REQUIREMENTS DESCRIBED IN IEC 61326-2-6; HOWEVER, THE ELECTROMAGNETIC ENVIRONMENT SHOULD BE EVALUATED PRIOR TO THE OPERATION OF THE INSTRUMENT.

IT IS THE OPERATOR'S RESPONSIBILITY TO ENSURE THAT A COMPATIBLE ELECTROMAGNETIC ENVIRONMENT FOR THE INSTRUMENT IS MAINTAINED, SO THAT THE INSTRUMENT PERFORMS AS INTENDED.

DO NOT OPERATE THE INSTRUMENT IN CLOSE PROXIMITY TO SOURCES OF STRONG ELECTROMAGNETIC RADIATION (E.G. UNSHIELDED INTENTIONAL RF SOURCES) AS THIS MAY INTERFERE WITH THE PROPER FUNCTION OF THE INSTRUMENT AND MAY ALSO LEAD TO INCORRECT RESULTS.

2. General

2.1 Intended Use / Introduction

The **labtech.com** LT-4500 instrument is a 96-well absorbance reader for the measurement of light absorbance (optical density) of liquid media. The instrument is intended to be used primarily in in-vitro diagnostic analysis of samples from the human body to obtain information on physiological and pathological states.

For applications in human medicine, only the LT-com software is intended for the use with the instrument. Software and instrument have been validated for measurement and for the evaluation of qualitative and quantitative Enzyme-linked Immunosorbent Assays (ELISA) according to the scheduled diagnostic parameters and instrument specifications; they are therefore intended for professional use in in-vitro diagnostics.

Note
If the LT-4500 absorbance reader or the LT-com software is modified in any way, the warranty will no longer be valid and the instrument will lose regulatory conformity.

Note
The operating authority must use only CE-labeled test kits for clinical diagnostic applications. The operating authority must assure that the combination of a particular CE-labeled test kit used with the CE-labeled LT-4500 absorbance reader and its options have been validated to meet the IVD directive 98/79/EC or other relevant national or local regulations.

If the LT-4500 absorbance reader is used differently from the 'Intended Use' mentioned above or if it is used with other software than LT-com, the instrument is no longer IVD conform and the user is responsible for the respective use and the necessary validation.

Note
Results obtained using the LT-4500 are influenced by the proper use of the instrument and microplates, according to the instructions given in this document, as well as the liquid compounds used (reagents, chemistry). The instructions for use, storage, and applications involving samples or reagents must be followed strictly. Results must therefore be interpreted carefully.

Note
Never open the housing of the instrument or the warranty will be rendered null and void.

The obtained transmission values are converted into optical density (OD) values according to the following formula:

$$\text{Transmission } T = \frac{I}{I_o}$$

I_0 = intensity of the incident light

I = intensity of the transmitted light

The OD is the logarithm of the reciprocal transmission.

$$OD = \text{Log}\frac{1}{T}$$

2.2 User Profile

2.2.1 Professional User - Administrator Level

The administrator is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

The administrator has extensive skills and is able to instruct the end user or the routine user in assay protocols in connection with a Labtech product within the bounds of the intended use.

Computer application skills and good English skills are required.

2.2.2 End User or Routine User

The end user or routine user is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

Computer application skills and good language skills for the respective national language at the installation site and English are required.

3. Getting Started

3.1 Unpacking and Inspection

3.1.1 Inspection of Delivered Packaging

The delivered instrument includes:

- External power supply
- Power cable
- USB cable for connection to external computer
- Instructions for Use (IFU), printed
- USB stick
  - Software (LT-com)
  - Instructions for Use (IFU), PDF files
  - Tools (e.g. Adobe Reader)

Note
To avoid undesired loss of data or virus/malware attack, never remove the write protection from the USB stick.

CAUTION
THE READER HAS BEEN TESTED WITH THE SUPPLIED USB CABLE. IF ANOTHER USB CABLE IS USED, THE CORRECT PERFORMANCE OF THE INSTRUMENT CANNOT BE GUARANTEED.

3.1.2 Unpacking Procedure

1. Visually inspect the packaging for damage before it is opened.
   Report any damage immediately.
2. Select a location to place the instrument. The location should be flat, vibration free, away from direct sunlight, and free from dust, solvents, and acidic vapors. Ensure that the distance between the instrument and the wall or other equipment is at least 5 cm.
3. Lift the instrument out of the carton and place it in the selected location. Take care when lifting the instrument.
4. Visually inspect the instrument for loose, bent, or broken parts.
   Report any damage immediately.
5. Compare the instrument's serial number on the bottom plate of the instrument with the serial number on the packing slip.
   Report any discrepancy immediately.
6. Check the instrument accessories against the packing note.
7. Save packing materials for further transportation purposes.

3.2 Power Requirements

The instrument is auto-sensing for the supplied voltage. Therefore, it is not necessary to make any changes to the voltage range. Check the voltage specifications and ensure that the voltage supplied to the instrument is correct according to the following specifications:

Voltage:	
Basic instrument with AC adapter:	100 – 240 V AC, 50/60 Hz
Basic instrument without AC adapter:	24 V DC

If the above mentioned voltage is not available in your country, please contact your local supplier.

Connect the instrument only to an electrical supply system with protective earth.

CAUTION
DO NOT USE THE INSTRUMENT IN AN INCORRECT VOLTAGE RANGE. IF THE INSTRUMENT IS SWITCHED ON WITH THE INCORRECT VOLTAGE IT WILL BE DAMAGED.

3.3 Environmental Requirements

The instrument should be placed on a flat, level surface that is free from dust, solvents, and acidic vapors.

Vibration and direct sunlight must be avoided to ensure correct results.

Ambient Temperature:	
Operation	15 °C to 35 °C (59 °F to 95 °F)
Storage	-30 °C to 60 °C (-22 °F to 140 °F)
Relative Humidity:	20 % to 80 % non condensing at operation temperature

3.4 System Requirements

3.4.1 Hardware requirements

The following requirements have to be met for using LT-com:

Hardware	Minimum	Recommended
Memory	512 MB	1024 MB
CPU	Pentium III or Atom	Pentium IV
Resolution	1024 x 600	1280 x 1024
USB ports	1 (USB 2.0 or higher)	2 (USB 2.0 or higher)

3.4.2 Software Requirements

The following requirements have to be met for using LT-com:

Operating System:	
Microsoft Windows	Windows XP Professional x86 SP2 Windows Vista Professional x86 SP1 Windows7 Professional 32 Bit, 64 Bit

Additionally supported software:
Microsoft Excel 2000
Microsoft Excel XP
Microsoft Excel 2003
Microsoft Excel 2007

3.5 Switching ON the Instrument

The following procedures detail the necessary steps required before switching on the instrument.

CAUTION
BEFORE THE INSTRUMENT IS INSTALLED AND SWITCHED ON, IT SHOULD BE LEFT TO STAND FOR AT LEAST THREE HOURS, SO THERE IS NO POSSIBILITY OF CONDENSATION CAUSING A SHORT CIRCUIT.

When the requirements mentioned above have been met, installation is carried out using the following procedure:

1. Connect the instrument to the external computer with the USB cable.
2. Ensure that the main power switch on the left side of the instrument is in the OFF position.
3. Insert the power cable into the main power socket in the left panel.
4. Switch the instrument on using the main power switch in the left panel.

The instrument is ready to measure microplates upon software installation.

Note

Before starting measurements make sure that the microplate position A1 is inserted correctly.

Microplates can only be measured without lids.

Close the plate transport cover before starting a measurement to avoid ambient light influencing the results.

3.6 Software

3.6.1 Introduction

The instrument control and data analysis software LT-com is delivered with the instrument. This version of LT-com is only compatible with the LT-4500 absorbance reader.

LT-com is a universal **reader control and data analysis software** for analyzing data generated by microplate tests using the labtech.com LT-4500.

LT-com offers all necessary functionality to become compliant with the FDA Regulation 21 CFR part 11 and with the European in vitro diagnostic directive 98/79/EC.

Note
It is important to note that the proper installation of the instrument and the LT-com software alone will not ensure compliance with laws and requirements. Corresponding policies concerning processes and standard operating procedures, including validation and quality control, must also be established.

3.6.2 Installation Procedure

To install the software, insert the USB stick to the USB port and proceed as follows:

1. LT-com installation wizard should start automatically and guide you through the installation process. If it does not, please run the file 'E:\Labtech.exe' (where E is the drive letter of the USB stick).
2. Select 'LT-com software' and - depending on the version you have ordered - 'Install LT-com' to start the installation procedure and follow the wizard.
3. Click **Install** to start the software installation procedure.
4. Click **I accept the terms of the license agreement** and **Next** to continue.
5. The **Customer Information** page appears: please enter user name and organization.
6. The **Configuration page** appears: choose the language.
7. **Use for regulated environments** page: click **Next** to continue.
8. Click **Install** to start the installation.
9. Click **Finish** to end the installation and to close the setup program.

The software can be started via the Windows **Start** menu by selecting **LT-com** in the program group 'Labtech'.

Note
It is very important that the person who installs the software has administrator rights on the computer.

By default, all file types associated with LT-com for LT-4500 are stored in corresponding subdirectories in the following directory:

...\All Users\Documents\Labtech\LT-com

3.6.3 Installation Qualification

Check successful installation of LT-com with the automatic installation qualification program:

Start *TecanIQ.exe* from the **Windows Start menu: Start > Programs > Labtech > LT-com IQ.**

Click **Check** to start the installation qualification. All installed components should have status **OK**. Please contact your local supplier if any potential problem is reported.

To close the installation qualification program, click **Cancel** or **Exit**.

Note
The installation qualification should be repeated each time LT-com is installed or updated to a newer version.

3.6.4 Start Working with LT-com

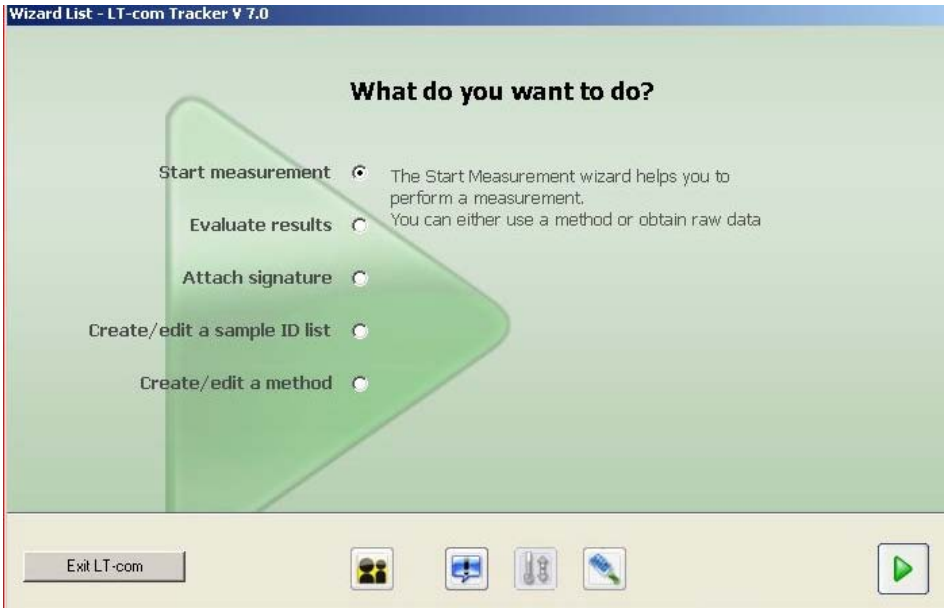
The main type of the user interface in LT-com is the wizard.

Standard LT-com wizards represent workflow modules, which are step-by-step guides for performing complex procedures.

Occasionally, menus are available in the heading bar. The **Menu** offers a conventional way of using the software: the relevant menu item is selected from the main menus. All subsequent actions are started instantly, or a dialog box is displayed where further selections or entries can be made.

User Interface – Wizard List

After launching LT-com, the **Wizard List** appears:



Each wizard can be started either by double-clicking or by selecting it and clicking the **Next**  button.

Start Measurement Wizard

The **Start Measurement wizard** includes the following options:

- **Obtain Raw Data** is used to generate raw data quickly and easily by setting the required measurement parameters and starting a measurement.
- **Run Strip Layout** is used to collect strips from different methods, combine the strips to one method and run this method.
- **Use Predefined Method** is used to perform measurements based on previously defined methods.
- **Start Favorite** is used to select one of the most frequently used methods from the list of numbered icons.

After the measurement is finished a workspace file is created.

Evaluate Results Wizard

The **Evaluate Results wizard** is used to view the raw data and to evaluate the results. The evaluation parameters can be viewed and data can be re-evaluated.

Attach Signature Wizard

The **Attach Signature wizard** is used to sign method and workspace files.

Create/Edit a Sample ID List Wizard

The **Create/Edit a Sample ID list wizard** is used to create new and to edit existing sample ID lists.

Create/Edit a Method Wizard

The **Create/Edit a method wizard** is used to define or edit methods.

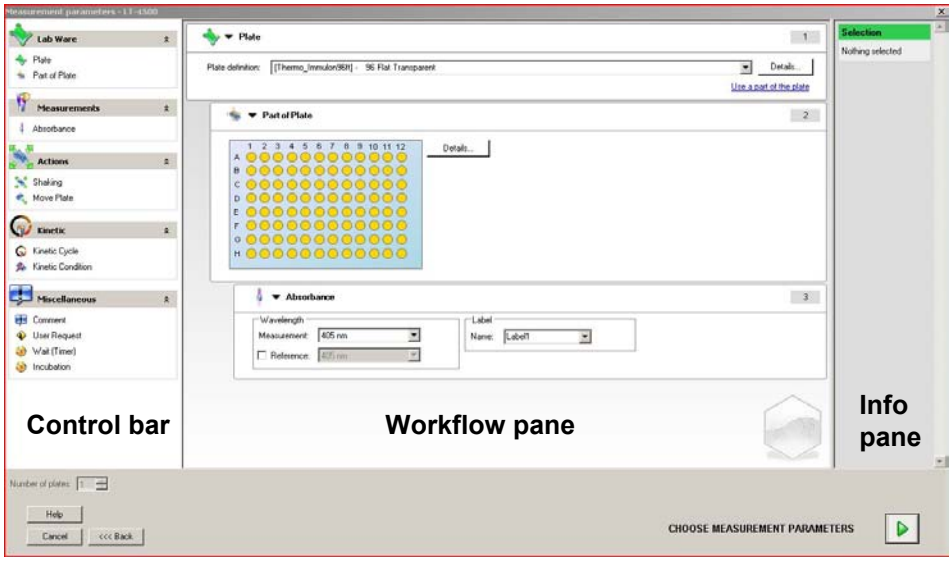
Note
For detailed information about the software please refer to the LT-com Instructions for Use.

Please note that some features (e.g. demo mode, licensing) described in the LT-com Instructions for Use might not be relevant (available). However, all necessary information is described in this IFU.

Note
Please find a detailed example of an ELISA measurement in chapter 6.

3.7 LT-com - Measurement Parameter Editor

The **Measurement Parameter Editor** is used to set up workflows. Each workflow is easily created by dragging and dropping the process steps into a sequence according to the application. The application workflow is then visible to the user in the workflow pane. Each process step (program element) can be copied and pasted (using the Windows standard shortcuts **Ctrl-C**, **Ctrl-V** or context sensitive mouse menu) and moved to the desired position in the workflow.



The **Measurement Parameter Editor** consists of the following items which are described in detail in the subsequent chapters:

- Control Bar
- Workflow Pane
- Info Pane

3.7.1 Control Bar

The **Control bar** is divided into five sections. Each section contains program elements used to create an individual workflow.

Create a workflow either by double-clicking the selected program element or by dragging and dropping it into the workflow pane.

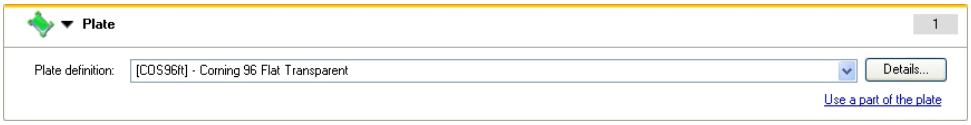
The following program elements are available when using an LT-4500:

Lab Ware	Plate Part of Plate
Measurements	Absorbance
Actions	Shaking Move Plate
Kinetic	Kinetic Cycle Kinetic Condition
Miscellaneous	Comment User Request Wait (Timer) Incubation

Lab Ware

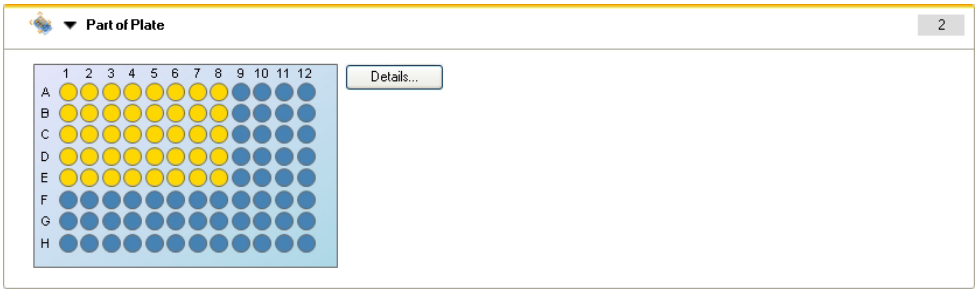
Plate

The **Plate** program element is used to select a plate format from the **Plate definition** drop-down list. Click **Details**... to see further information on the selected plate.



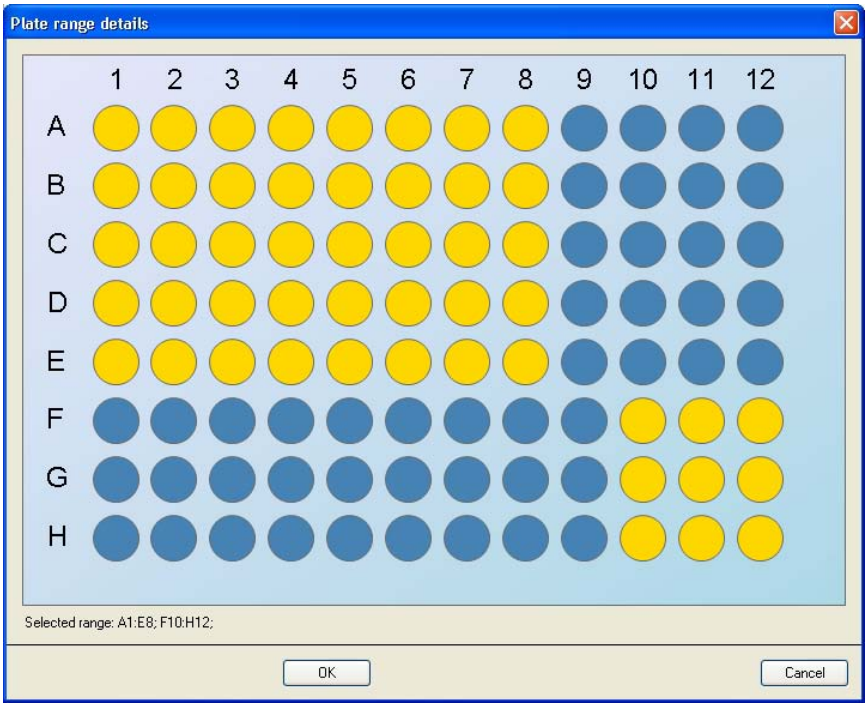
Part of Plate

By default the **Part of Plate** program element is collapsed. When expanded by clicking ▶ it displays a 96-well microplate. To measure individual wells, click the desired well or to measure a range of wells drag a frame around the desired range. Clicking on **Details** , the plate preview can be zoomed.



Independent Parts of Plate

Independent parts of the plate can be selected:



A second range of wells can be selected by pressing the **Control key** on the keyboard and dragging a frame over the wells to be selected.

Measurements

Absorbance

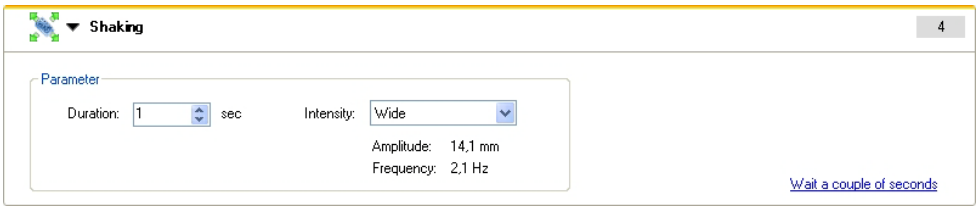
The **Absorbance** program element is used to perform absorbance measurements. Enter or select the respective parameters.

Two drop-down lists display the available measurement and reference filter wavelengths, according to the inserted absorbance filters. If the drop-down lists are empty, the filters have not been defined.

Actions

Shaking

Select the **Shaking** program element if the plate is to be shaken, either before the measurement or between kinetic cycles.



Enter the respective parameters:

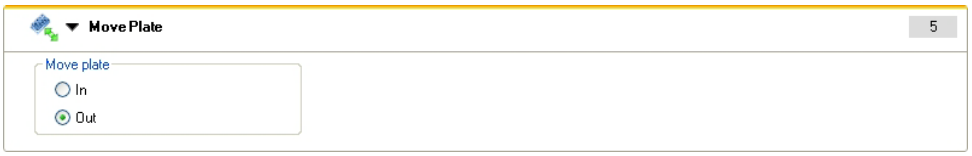
Duration	Enter the duration of the shaking process.
Intensity	Enter the desired shaking mode. Amplitude and frequency are displayed when choosing the respective shaking mode.

See chapter 4.1.1 for available shaking modes.

Clicking the link Wait a couple of seconds inserts a new program element.
See page 26 for details.

Move Plate

Select the program element **Move Plate** to move the plate out of or into the instrument at a certain moment during the workflow.

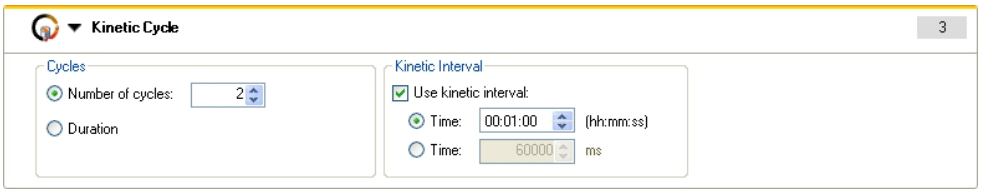


If the plate is moved out of the reader during a workflow (e.g. to pipette some liquid into the wells of the microplate), it must be followed by a subsequent move plate **In** step, so that the measurement can be finished.

Kinetic

Kinetic Cycle

Use the program element **Kinetic Cycle** to perform several consecutive measurements, which may be executed in certain intervals.



Enter the respective parameters:

Cycles	**Number of cycles**: Enter a number or click the up or down arrows for the number of actual measurement steps (2 – 1000 cycles) **Duration**: Enter the duration, format hh:mm:ss.
Kinetic Interval	**Use kinetic interval**: Enter the time interval (hh:mm:ss or ms).

Kinetic Condition

Use the **Kinetic Condition** program element to define which actions should be executed at a certain cycle.

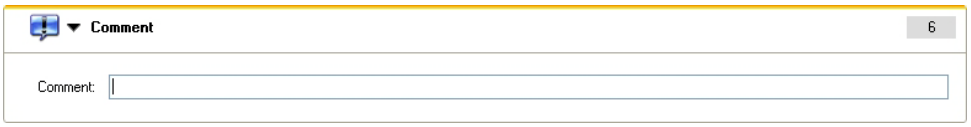
If **2** is entered for **Execute command at cycle** within a kinetic measurement containing, e.g. a **Shake** step, shaking is performed only at cycle 2.

Note
Kinetic conditions such as Shake should be inserted right after a Kinetic Cycle program element in order to ensure optimal result reproducibility. Users are advised to set up suitable scripts prior to the measurements and to use the same script for all similar kinetic measurements in order to obtain comparable results.

Miscellaneous

Comment

Use the program element **Comment** to enter a remark or statement for the current measurement in the text field.



User Request

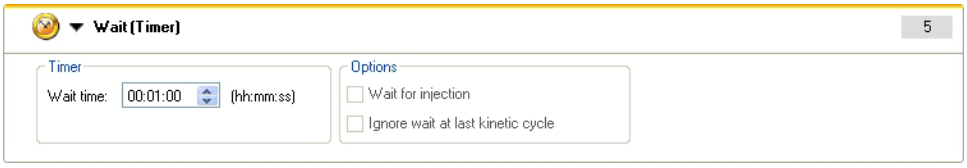
The **User Request** program element informs the operator of the instrument to execute a definite action during the workflow at a certain time.

If for example the **Move Plate** program element is used to move the plate out to perform a certain action, then the entered text should inform the operator to perform these actions. A dialog box shows the message and the measurement process stops until **OK** is clicked.

Wait (Timer)

Use the **Wait (Timer)** program element to define a certain waiting period before the next step within a workflow is executed.

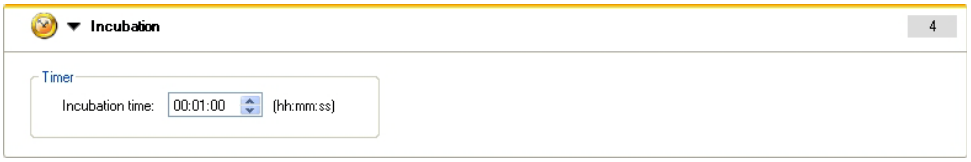
In the **Wait time** field enter the required time.



Enter the respective parameters:

Timer	Enter the Wait time (hh:mm:ss)
Options	Ignore wait at last kinetic cycle: When the program step Wait (Timer) is the last action within a kinetic run, the wait time will be ignored in the last cycle.

Incubation



Enter the appropriate parameters for incubation:

Incubation time	Enter the total time (min. 5 s)

3.7.2 Workflow Pane

The **Workflow pane** is the window, where the measurement script is visible and where parameters are defined and edited.

There are two ways to insert a program element from the **Control bar** into the **Workflow pane**:

- Select a program element from the **Control bar**; by double-clicking it, it is inserted into the **Workflow pane** directly after the previous program element.
- Click the program element in the **Control bar** and drag it into the **Workflow pane** to the respective position.

The program elements are numbered according to their sequence.

Once a program element has been inserted into the **Workflow pane**, settings and parameters for this element can be entered or edited.

Single program elements inside the **Workflow pane** can be collapsed to display the most important information or expanded to access all editable functions. Click one of the triangles next to the title of the program element, ▼ or ▶, to switch between the two view modes.

By default, the measurement parameter editor starts with the **Plate** element, the **Part of Plate** element (collapsed) and an **Absorbance** element in the **Workflow pane**.

Currently selected program elements within the **Workflow pane** are displayed with a yellow line on the upper border.

If a program element contains errors or is invalid within the current workflow, the element will be flagged with an error mark and the number of the element is highlighted in red. In the **Info pane** detailed information on the error is displayed. If the workflow contains errors, the measurement parameters cannot be chosen.

Hierarchy of Elements

The hierarchy of elements in the **Workflow pane** is as follows:

1. Plate
2. Part of Plate (Range)

Any desired measurement step can be inserted directly after a plate or range element. Use **Release** and **Indent** to modify the sequence of execution of the single strip component. Select an element in the **Workflow pane**, click the right mouse button and select **Release** or **Indent**.

Other elements from the **Control bar** can be inserted into the hierarchy of a workflow as follows:

The first **Range** element is inserted directly after the **Plate** element; then all subsequent **Range** elements can be inserted.

Kinetic steps are possible within a **Plate** or **Range** element.

User Request, **Comment** and **Wait** steps are possible within a **Plate** or **Range** element.

3.7.3 Info Pane

The **Info pane** on the right side of the screen displays information that is relevant for the currently selected program element. Any warnings and errors are shown.

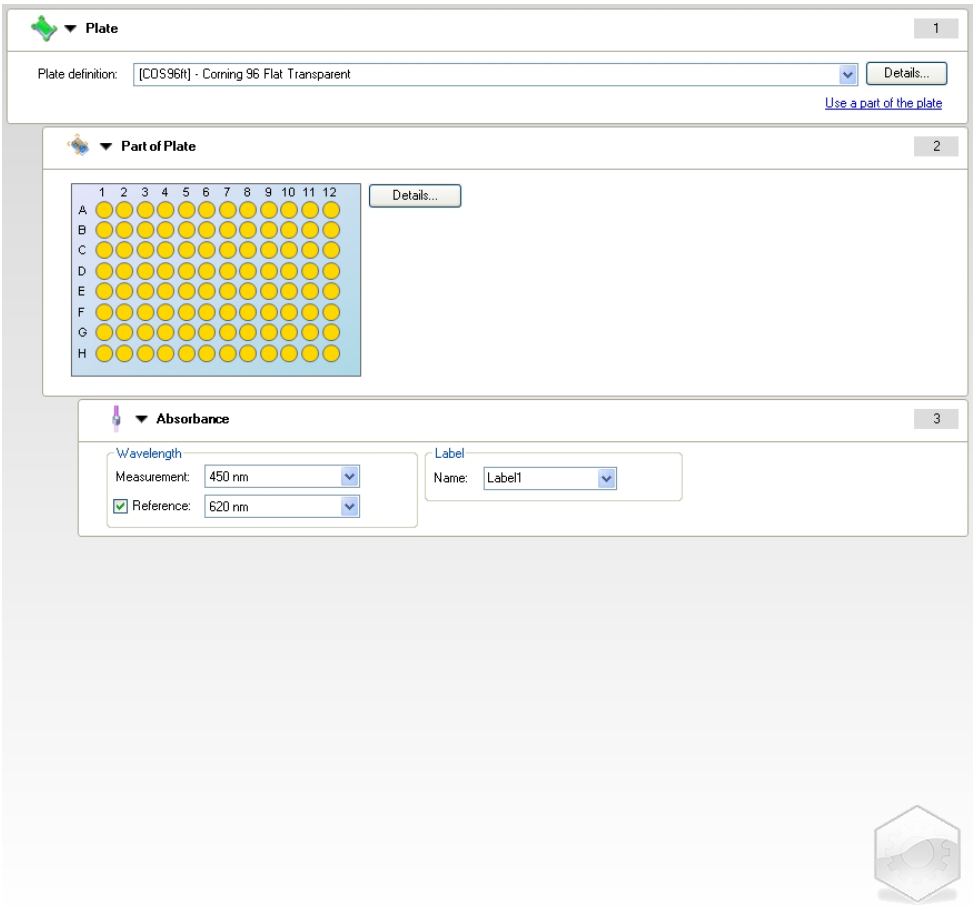
3.8 LT-com - Defining Measurements

The following chapter describes some examples to illustrate the definition of different measurements.

3.8.1 Defining Endpoint Measurements

The following example describes an **Absorbance Endpoint Measurement** in all wells of a 96-well microplate.

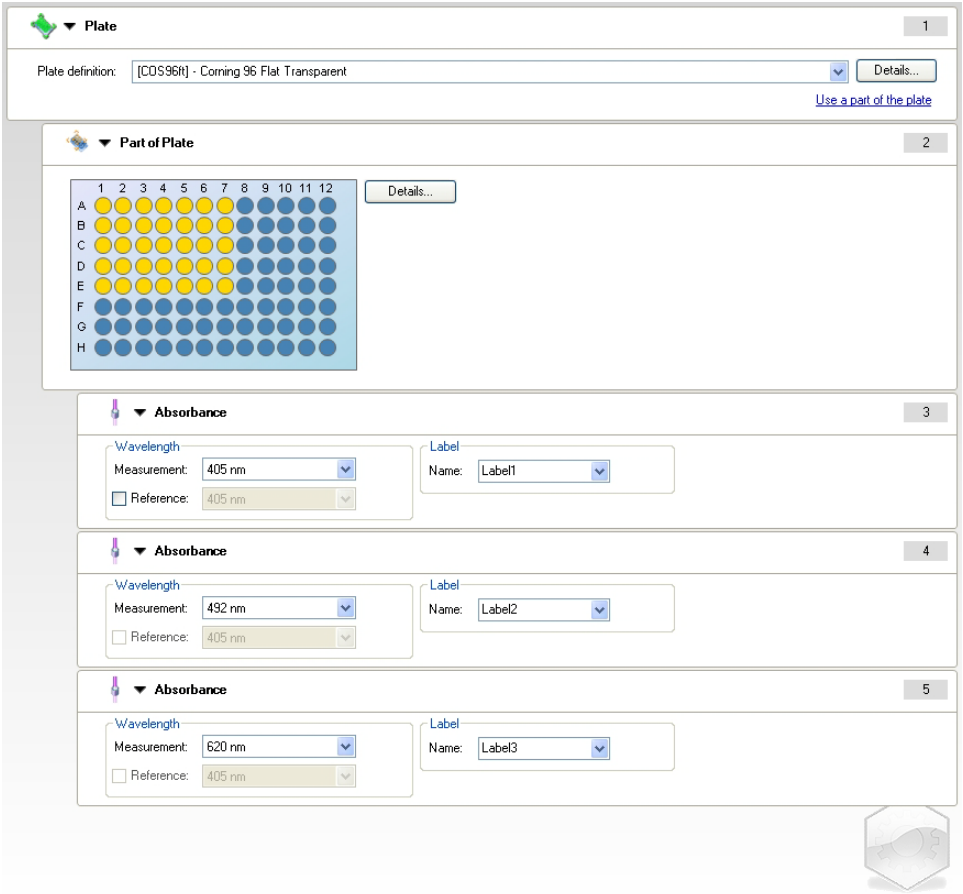
1. Select a 96-well microplate from the **Plate definition** drop-down list.
2. By default, all wells of the 96-well microplate are chosen for measurement.
3. Enter the desired measurement and reference wavelengths.



3.8.2 Defining Multilabel Measurements

The following example describes an **Absorbance Multilabel Measurement** in a defined range of a 96-well microplate (A1:E7). Three absorbance labels shall be measured.

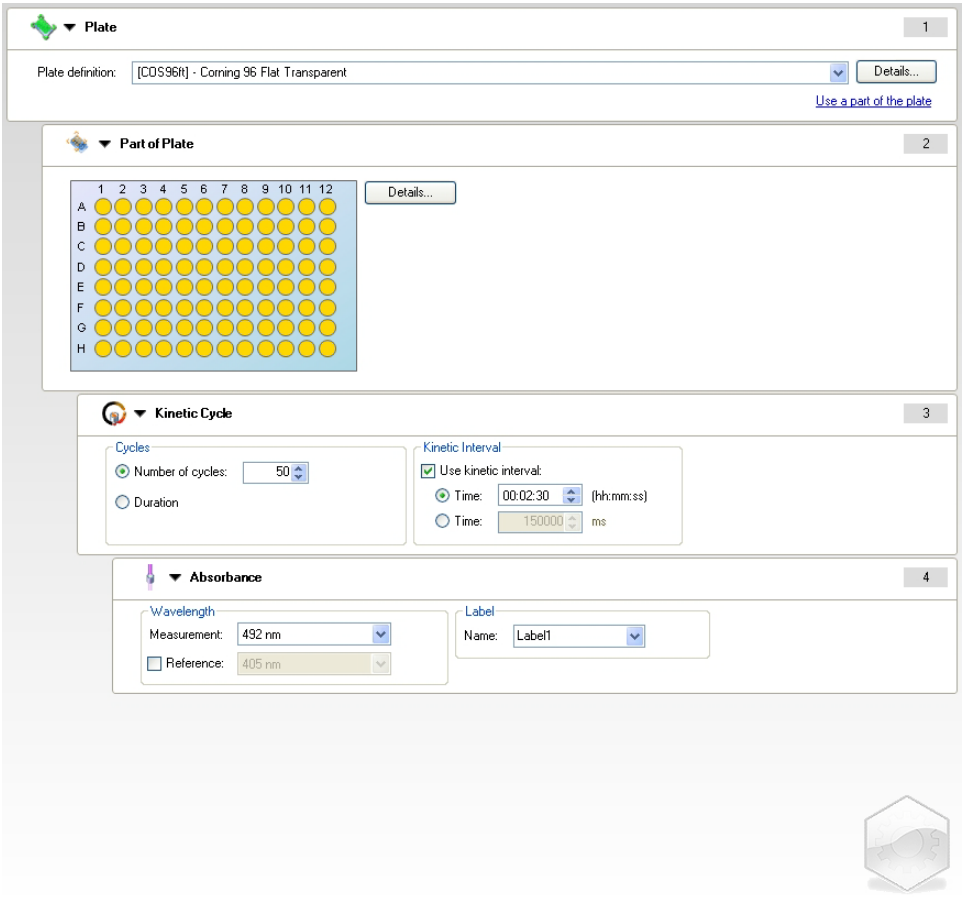
1. Select a 96-well microplate from the **Plate definition** drop-down list.
2. By default, all wells of the 96-well microplate are chosen for measurement.
   Click ▶ to expand the **Part of Plate** element. Thereafter, select the desired plate range (A1:E7).
3. Enter the desired measurement wavelength.
4. Insert 2 more **Absorbance** elements and enter the measurement wavelengths.



3.8.3 Defining Kinetic Measurements

The following example describes a kinetic measurement of a 96-well microplate.

1. Select a 96-well microplate from the **Plate definition** drop-down list.
2. Insert a **Kinetic Cycle** program element between the part of plate and the absorbance element.
3. Cycles/Number of cycles: 50
4. Kinetic interval (interval between measurements): select **Use kinetic interval** and enter: 2 minutes 30 seconds.
5. Define the **Absorbance** element by entering the desired measurement wavelength.



3.8.4 Indenting and Releasing Program Elements

The decision to indent/release a program element will modify the workflow of the instrument during measurements.

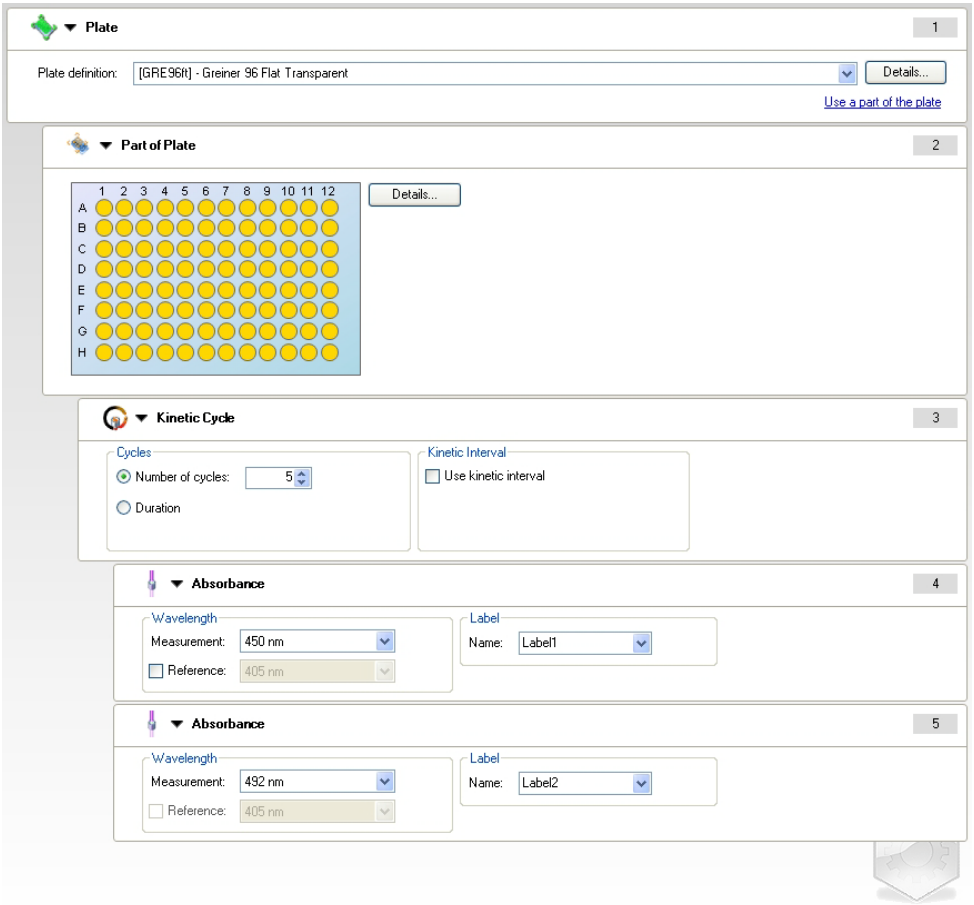
The actions of all program elements with the same indentation are performed sequentially. The only dependence between these program elements is that the next action starts directly after the previous action is finished.

A program element that is indented more than the previous program element shows dependence between the two program elements. This means the parameters defined in the first program element are also active for the second (indented) program element.

The following is an example of how to define a **Multilabel kinetic** with two **Absorbance labels**. The example shows that the two **Absorbance** program elements depend on the **Kinetic Cycle** program element, which depends on the **Part of Plate** program element, which depends on the **Plate** program element. Define the parameters for an example as follows:

1. **Plate**: e.g. Greiner 96 Flat Transparent
2. Kinetic Cycle/Number of cycles: 5
3. Absorbance/Wavelength Label 1: 450 nm
4. Absorbance/Wavelength Label 2: 492 nm

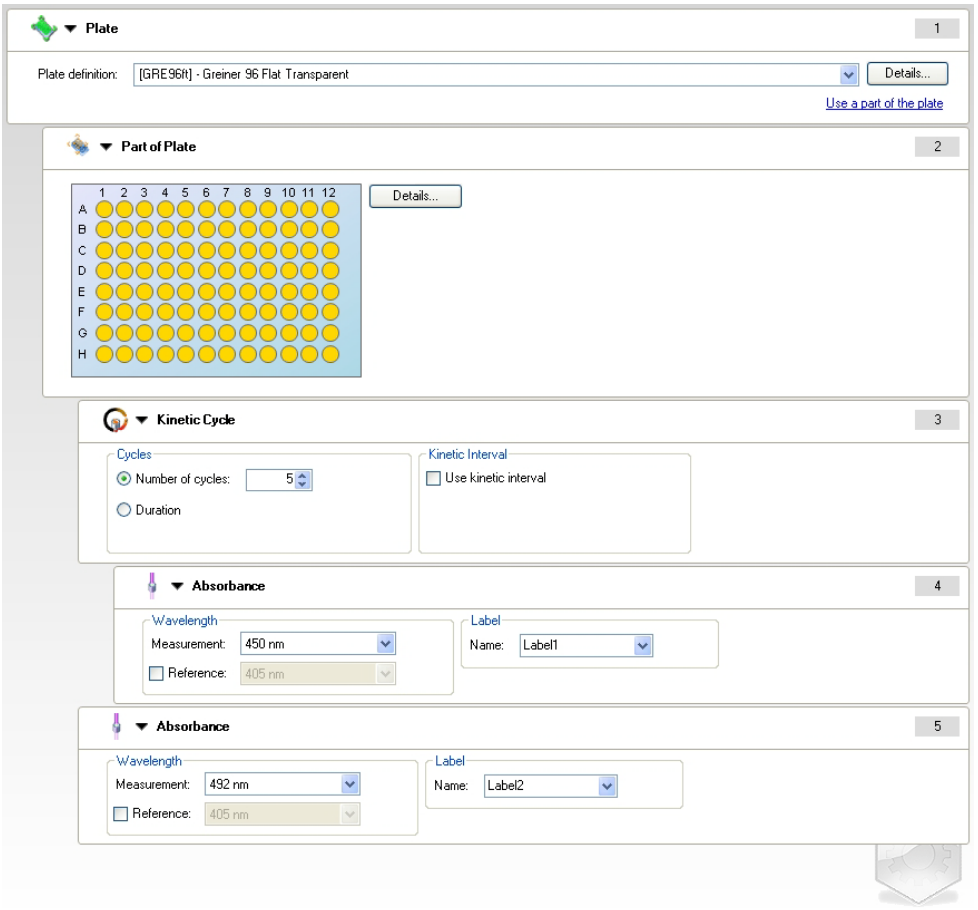
The **Workflow pane** appears as shown in the screenshot:



The definition above results in the following workflow:

The absorbance of all wells of the 96-well microplate is first measured at 450 nm and then at 492 nm. Both absorbance measurements are performed in 5 kinetic cycles.

Releasing the second **Absorbance** program element, so that it is aligned with the **Kinetic Cycle** element, changes the workflow. Select the second **Absorbance** program element and click the right mouse button. Select **Release Strip** from the context sensitive menu. The **Workflow pane** appears as shown in the following screenshot:



In this workflow, an **Absorbance kinetic** measurement with 5 cycles is done for the first absorbance at 450 nm; finished this loop, **Absorbance endpoint** measurement at 492 nm is performed.

3.9 Optimizing for Best Performance

The instrument has been fully factory tested to ensure that its performance is within the specified limits (see 4.4.2 for details).

The greatest accuracy can be obtained from the instrument by observing the recommendations mentioned below:

3.9.1 Instrument Location

The instrument should be positioned in an appropriate place (see chapter 3.3 for detailed information).

3.9.2 Operating Procedure

General

- It is recommended to follow the standard operating procedures for the assays used.
- The best reproducibility is obtained, when the measurement wavelength corresponds to the maximum absorbance wavelength of the particular solution.
  It is important to use the maximum absorbance wavelength, if the absorbance curve of the sample is over a narrow wavelength band. Please be aware that measurements in the slope of an absorbance peak will limit the accuracy of OD values.
- After each microplate has been measured, please refer to the test kit package for information regarding the validation procedure.
- Use the recommended absorbance filters for the LT-4500.

Microplates

- The instrument can be used with those types of microplates which are described in chapter 4.4.3. The best results are obtained when flat bottom microplates are used. Depending on the type of microplate being used, the measurement results may vary.
  Take care especially when using microplates with C-, U- or V-shaped bottom or strip-well plates because it is possible that the measurement results might differ to the specifications described in this document.
  Make sure that the type of microplate used with the LT-4500 absorbance reader is suitable for the respective application.
- Use only perfectly clean microplates.
- Do not allow dust to settle onto the solutions or the microplate during an incubation period prior to the measurement.
  It is recommended to use a cover for protection.
- Inaccuracies in the amount of solution pipetted have a greater effect on the results obtained, when small amounts of solutions are used.
- The form of the meniscus of the solution can cause inaccuracies in the results, particularly if small amounts of solution are used.

4. Instrument Features

4.1 Instrument Features

The following absorbance measurement modes are available on the LT-4500: endpoint, kinetic, and multilabel measurements.

4.1.1 Microplate Shaking

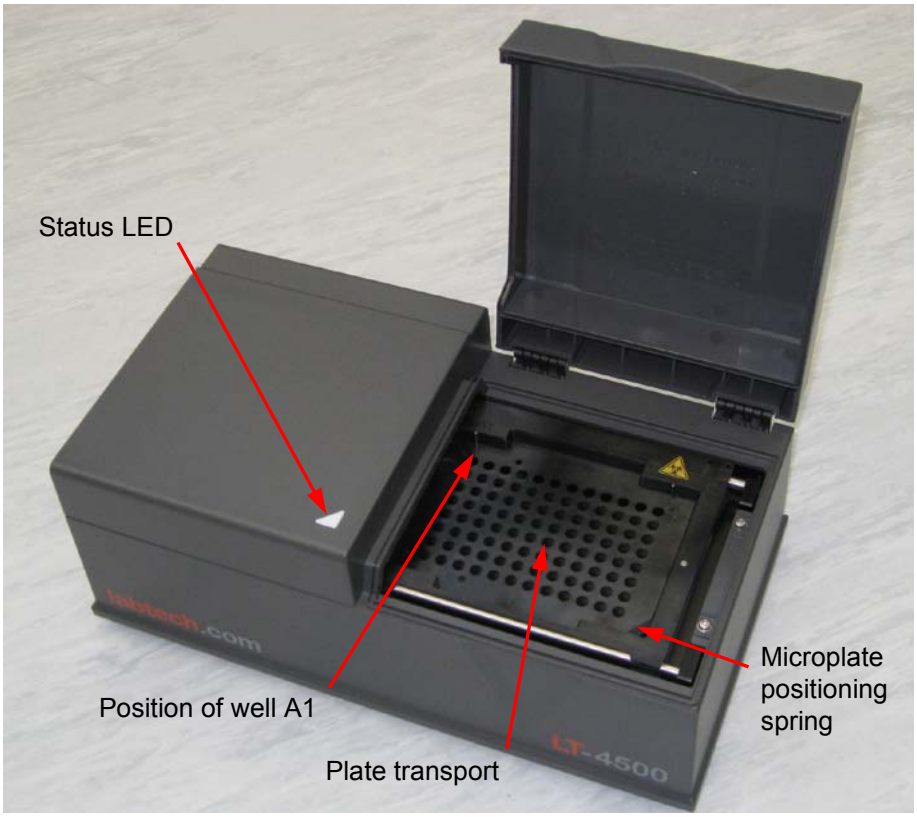
The LT-4500 is able to shake the microplate before it is measured. The microplate can also be shaken between each of the kinetic measurement cycles. Use LT-com to set the shaking mode.

CAUTION
WHEN SHAKING MICROPLATES, SPILLAGE MAY OCCUR IF THE WELLS ARE FILLED WITH A TOO HIGH VOLUME.

Shaking modes for the LT-4500:

Shake Mode	Shake Width	Shake Frequency
HIGH	2.8 mm	12.3 Hz
NORMAL	4.4 mm	9.2 Hz
LOW	4.4 mm	7.8 Hz
WIDE	14.1 mm	2.0 Hz

4.2 Instrument Description



The illustration above shows the components of the instrument.

The status LED gives information of the status of the instrument:

- **Green blinking**: instrument is not connected to LT-com
- **Green**: instrument is connected and ready for measurement
- **Red**: measurement is in progress

On the left panel of the instrument the USB port, main power switch, and main power socket can be found.

The nameplate is attached to the bottom of the instrument.

Example Name Plate

:

Contents of the name plate (e.g. model name and article number) may vary depending on the specific model.

For an overview of the various instruments for which these Instructions for Use are valid, see the Declaration of Conformity on the last page of this document.

4.3 Filter Wheel Description

The LT-4500 standard filter wheel is delivered with four narrow band interference filters that have a fixed wavelength (405, 450, 620, and 492 nm). It is possible to equip the filter wheel with up to 8 filters. For accessorily available filters please contact your local supplier.

The filters of the standard filter wheel are mounted as listed below:

Filter position	Filter wavelength
1	405 nm
2	450 nm
3	620 nm
4	492 nm
5 - 8	empty filter positions

When a wavelength is selected for measurement the specific filter is brought into the light beam by moving the filter wheel to the according position.

Note
For more information about the definition of a new filter see 7.5.2.

4.4 Instrument Specifications

The tables below list the specifications for the LT-4500 absorbance reader.

4.4.1 General Specifications

PARAMETERS	CHARACTERISTICS
Main power input External power supply	Power supply: Basic instrument with AC adapter: 100-240 V AC, 50/60 Hz, max. 1.2 A (auto sensing, Over voltage category II) Basic instrument without AC adapter: 24 V DC (Over voltage category I)
Consumption LT-4500	Standby mode: approx. 12 VA Operational mode: max. 30 VA
Outside dimensions	Width: 34.7 cm (13.66 inch) Depth: 18.9 cm (7.44 inch) Height: 13.4 cm (5.28 inch)
Weight	2.6 kg (power supply included)
Ambient temperature:	
Operation	15 °C to 35 °C (59 °F to 95 °F)
Storage	-30 °C to 60 °C (-22 °F to 140 °F)
Relative humidity	20 % to 80 %
Pollution degree	2
Method of disposal	Contaminated waste
Environment	See chapter 3.3 for more information.

4.4.2 Measurement Specifications

PARAMETERS	CHARACTERISTICS
Measurement time: single wavelength dual wavelength	 < 15 seconds < 20 seconds
Wavelength range: Standard	 400 - 750 nm
Measurement range: 400 - 750 nm	 0 - 4.000 OD
Resolution:	0.0001 OD
Accuracy: 450, 492 nm 0.000 - 2.000 OD 2.000 - 3.000 OD	 ≤ (1.0 % + 0.010 OD)* ≤ (1.5 % + 0.010 OD)*
Precision: 450, 492 nm 0.000 - 2.000 OD 2.000 - 3.000 OD	 ≤ (0.5 % + 0.005 OD)* ≤ (1.0 % + 0.005 OD)*
Linearity: 450, 492 nm 0.000 - 2.000 OD 2.000 - 3.000 OD	 ≤ 1 % ≤ 1.5 %
Wavelength selection: Standard filter	Narrow bandwidth interference filters. Up to 8 filters can be mounted in a filter wheel.
Filter wavelength accuracy:	Central wavelength ± 2 nm
Filter bandwidth: At 50 % transmission	 10 ± 2 nm
Light source:	LED
Computer interface: USB port	 USB 1.1 / 2.0
All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety and equivalent local standards.	

* better than or equal to x % of measurement value plus corresponding OD value.

4.4.3 Microplates

All 96-well microplates with transparent bottom (flat, C-, U-, and V-shaped; including strip-well microplates) that are conform to the following standards can be used with the LT-4500 absorbance reader:

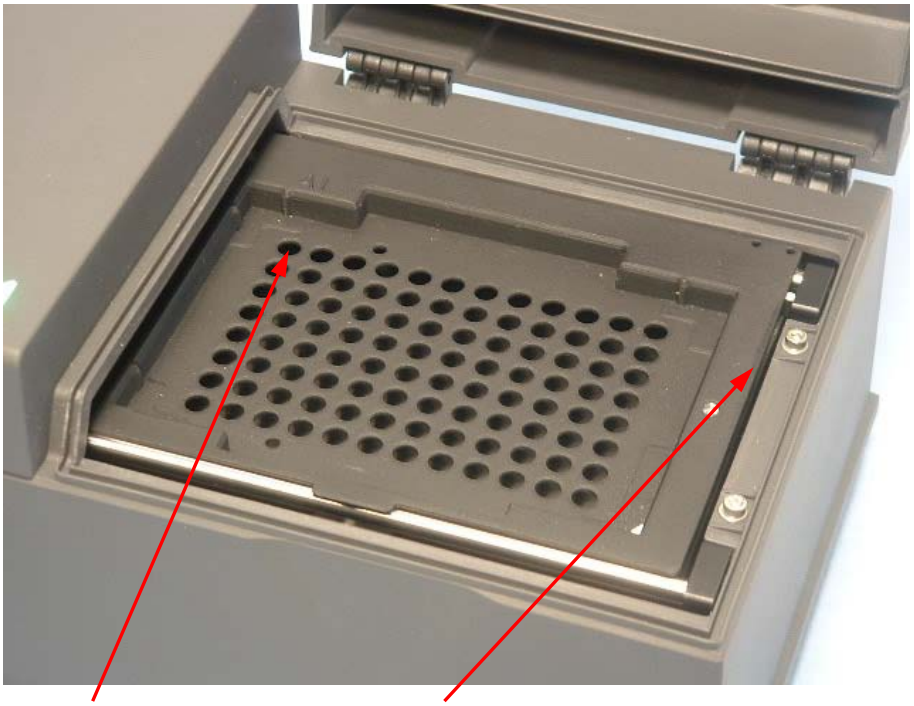
ANSI/SBS 1-2004; ANSI/SBS 2-2004; ANSI/SBS 3-2004; ANSI/SBS 4-2004

CAUTION
ONLY USE MICROPLATES WITHOUT LIDS AND DO NOT USE MICROPLATES HIGHER THAN 15.2 MM.

Handling the Microplate

Insert or remove the microplate only when the plate transport is fully ejected (as illustrated below) and the plate transport motor is not active. Do not open the lid when the Status LED shines red.

WARNING
ALWAYS WEAR DISPOSABLE GLOVES AND PROTECTIVE CLOTHING WHEN HANDLING THE MICROPLATE.



Position of well A1 Plate transport – fully ejected

4.5 Available Option for LT-4500

4.5.1 LIS Option – Handheld Barcode Scanner

The LIS option (Laboratory Information System Option) is an additional option for LT-4500 instruments and allows reading of plate and sample IDs from compatible barcodes by using a handheld barcode scanner which is part of the LIS Option.

For ordering information please contact your local supplier.

The LIS Option consists of:

- A dedicated pre-adjusted handheld barcode scanner
- USB cable for the scanner

The handheld barcode scanner allows reading of plate and sample IDs from compatible barcodes (see below).

LT-com supports the transfer of all data including results and sample IDs to a pre-defined location in a LIS system using the high-level data transfer standards according to ASTM E1394.

It is possible to export data in the following file formats:

- *.xls
- *.txt
- ASCII
- ASTM

Barcode Scanner Test

It is recommended to check the functionality of the handheld barcode scanner in regular terms. Therefore, a software tool is available online. Please contact your local supplier for further information.

Operating the Handheld Barcode Scanner

LED **ON** means: correct reading of barcode

LED **OFF** means: ready to perform barcode reading

LED **blinking** means: start-up check in progress

The dedicated handheld barcode scanner, which is part of the LIS-option, scans barcodes **on contact**. To read a barcode the trigger button on the handheld barcode scanner must be pressed.

Barcode scanning is performed along an imaginary line passing across the reading window. This imaginary line must pass through the entire code.

Successful scanning is obtained by keeping the handle of the handheld barcode scanner parallel to the code surface.

CAUTION
TO GUARANTEE RELIABLE OPERATION IT IS MANDATORY TO USE ONLY THE HANDHELD BARCODE SCANNER SUPPLIED WITH THE LIS OPTION IN COMBINATION WITH THE LT-4500 READER. THE CHANGING OF PREDEFINED SCANNER SETTINGS IS NOT ALLOWED.

Supported Barcode Types

The following barcode types are suitable for processing by the handheld barcode scanner:

- Codabar
- Code 128
- Code 39
- Interleaved 2 of 5

The maximum barcode length which can be processed by the handheld barcode scanner supplied with the LIS option is 20 characters.

Note
The barcode label must be of good printing quality with clearly separated individual barcode lines.
Dirty, folded, wet, or damaged barcode labels must not be used. The adhesive labels must be flat and not peeling off at the edges.

We recommend ensuring the quality of the barcode labels by means of local SOPs.

PARAMETERS	CHARACTERISTICS
Reading field	80 mm typical (3.150 inch)
Max. resolution	0.13 mm (5.118 mil; 0.005 inch)

4.6 Instrument Accessories

The list below contains the available optional accessories for the LT-4500, which can be ordered additionally:

- LIS Option - handheld barcode scanner
- Supplementary filters
- Filter assembly tool
- MultiCheck™ plate for LT-4500

For further information and availability in your country please contact your local supplier.

5. Quality Control

5.1 Introduction

CAUTION
IF AT ANY TIME THE ANALYTICAL PERFORMANCE OF THE LT-4500 IS IN QUESTION, FOLLOW THE INSTRUCTIONS GIVEN FOR QUALITY CONTROL OR CONTACT YOUR LOCAL SUPPLIER.

This chapter provides information about the self-check procedure for the instrument and instructions on how to easily check the operational quality.

5.2 Self Check Procedure

During the connection of the LT-4500 to the LT-com reader control software, motors and sensors are checked and plate carrier and filter wheel are initialized.

Prior to each measurement a self check calibration procedure is performed to ensure that the instrument is working correctly and to calibrate the optical system.

5.3 Operational Qualification (OQ)

The following tests can be performed to ensure that the instrument is working correctly and accurate results are being obtained.

The reproducibility and accuracy of the instrument may vary with the type of solution and microplate used.

To eliminate this effect, the instruments are tested in the factory with a calibration plate, which removes the influence of the solution and any variation due to the positioning of the microplate when it is being measured.

5.3.1 MultiCheck Test

The MultiCheck test provides an automated check of reader performance including accuracy, linearity, precision, and alignment with NIST traceable standards.

5.3.2 Microplate Test

If the optical densities of the wells in the microplate are not consistent, the results obtained with this type of microplate will be influenced.

This inconsistency can be checked by reading an empty microplate.

The OD values obtained from the measurement of the empty microplate should be in a narrow range. For example: ± 0.010 OD.

If the OD values are not within this range this type of microplate should not be used.

By using dual wavelength measurements, the influence of the difference in OD values of the microplate is removed or reduced to a level that is within acceptable limits.

5.3.3 Instrument Precision with Liquid Samples

This procedure can be used to check the reproducibility of the measurements. The use of a microplate with flat bottom is recommended.

Fill a new microplate with a freshly prepared Orange G solution; use different dilutions of the solution in each well so that a range of optical densities is obtained. Make sure that the wells contain at least 200 µl. The dilution series should be within the range of 0.1 to 3.0 OD. To reach about 3 OD it is recommended to use 125 $mg.l^{-1}$ Orange G (Sigma, Cat. No. O7252).

Define a test run using the 492 nm filter and then measure the microplate at least three times.

For each well calculate the:

- average OD value
- standard deviation

Example

Readings 0.000 to 2.000 OD

The standard deviation of each well should be within (0.5 % + 0.005 OD).

Calculation of maximum allowed deviation using 1.000 OD as average OD value:

1.000 * 0.5 % + 0.005 = 0.010 OD

Readings 2.001 to 3.000 OD

The standard deviation of each well should be within (1.0 % + 0.005 OD).

Calculation of maximum allowed deviation using 2.400 OD as average OD value:

2.400 * 1.0 % + 0.005 = 0.029 OD

Readings above 3.000 OD

Readings above 3.000 OD are only used as an indication and the precision cannot be guaranteed.

5.3.4 Instrument Linearity with Liquid Samples

The linearity for the instrument and application at the wavelength used can be checked by using a dilution series of a solution.

The result depends on the purity of the dye used and the meniscus of the liquid in the wells.

As a reference, a dilution series of Orange G solution for measurements at 492 nm can be used.

The dilution series should be within the range of 0.1 to 3.0 OD. To reach about 3 OD it is recommended to use 125 mg.l^{-1} Orange G (Sigma, Cat. No. O7252).

For other wavelengths, different solutions must be used.

200 µl of each dilution are then pipetted into the microplate, a minimum of at least two samples should be used for each dilution to reduce the errors caused by pipetting.

The microplate is then measured and a linear regression of OD against concentration is drawn from the average of the measured OD values.

Determine the unweighted residual square value R^2 of the regression line.

Typical residual square values for a standard application are equal or better than $R^2 = 0.998$.

Note
Data can vary due to pipetting inaccuracy.

6. Application Example

6.1 Introduction

The LT-com ***example files*** provide LT-com methods and workspaces to introduce the software and to ease the user's work with it. The example files for a quantitative and a qualitative ELISA assay are installed automatically upon installation of LT-com.

6.2 Step-by-Step Example: Quantitative ELISA

A step-by-step example (quantitative test) of how to create a method in LT-com is provided in this chapter. By following the instructions you will learn how to define evaluations from a test kit description in LT-com

Note
These example files are available in the default data path and must be converted.

6.2.1 Test Kit Description

In the manufacturer's test kit description of a quantitative IgM – Antibody detections – ELISA the following instructions are found: Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	C3	S1									
B	NC	C4	S2									
C	NC	C4	S2									
D	C1	C5	S3									
E	C1	C5	S3									
F	C2	C6										
G	C2	C6										
H	C3	S1										

BLK = Blank, NC = Negative control, C1 - C6 = Calibrators (Standards), S1 - S = Samples

Measurement and Evaluation

Read plate at a wavelength of 492 nm, reference at 620 nm.

Blank reader/plate on well A1.

Concentrations of the Calibrators (Standards):

Calibrator 1	5 UA/ml
Calibrator 2	10 UA/ml
Calibrator 3	20 UA/ml
Calibrator 4	40 UA/ml
Calibrator 5	80 UA/ml
Calibrator 6	160 UA/ml

After the blank correction the optical densities (OD 492 - OD 620) are plotted versus the concentration. The regression line that goes through these points is the standard curve.

Interpretation of the test results:

IgM < 18 UA/ml	Negative
18 UA/ml ≤ IgM < 22 UA/ml	Intermediate
IgM ≥ 22 UA/ml	Positive

The calculated IgM concentration of both negative controls must be under 8 UA/ml.

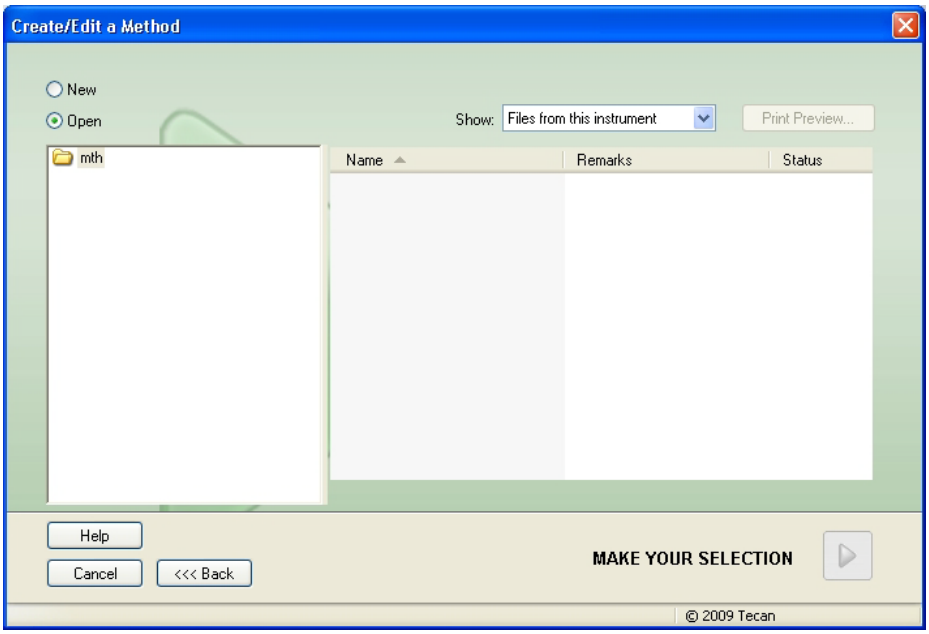
Data Handling

After the measurement, the data file (workspace) is stored automatically and a report containing the measurement parameters, plate layout, blanked values, standard curve, IgM-concentrations, cutoff definition, qualitative results of the samples and validations is created.

Additionally, the layout and the qualitative results are being stored as ASCII file.

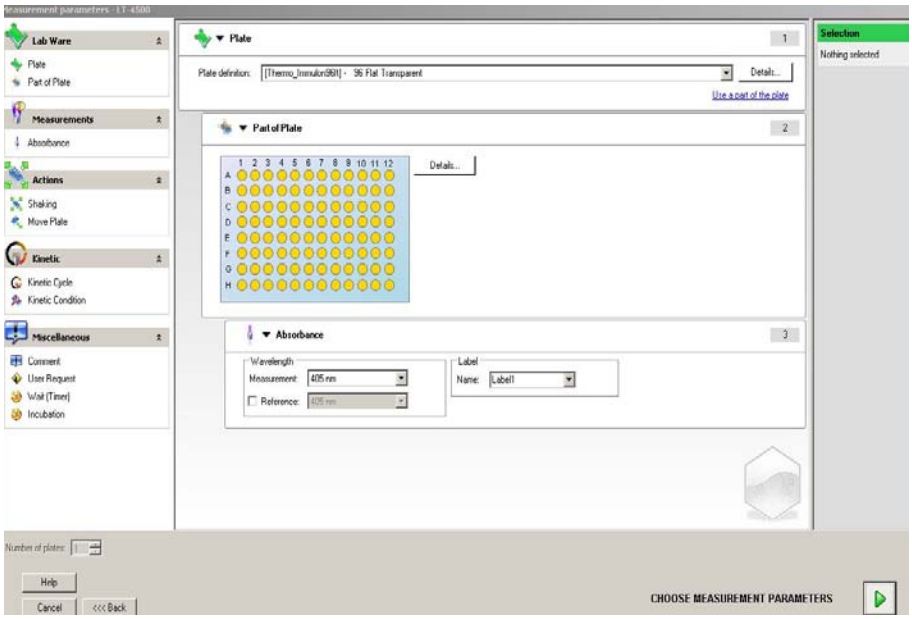
6.2.2 Create a Method

In the **Wizard List** dialog box, select **Create/edit a method** and click **OK**. Click **Continue** on the **Welcome** page of the **Create/edit a method wizard** and the **Select a file** dialog box appears. Select **New**.

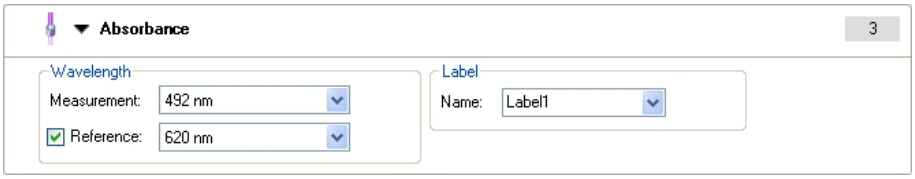


Measurement Parameters

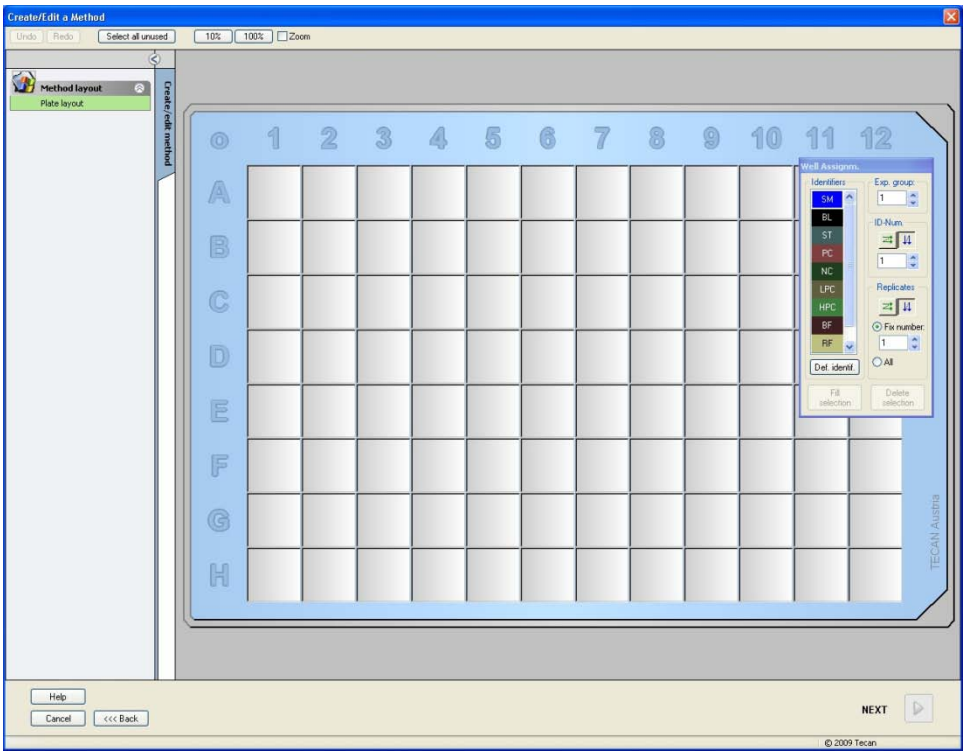
Click **Make Your Selection** and the **Measurement parameters** page appears.



On the **Wavelength** strip select 492 nm as Measurement wavelength and 620 nm as Reference wavelength.

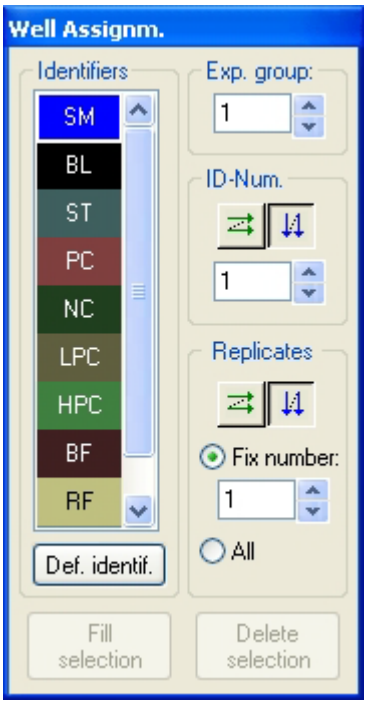


Continue Wizard by clicking **Choose measurement parameters** and the **Plate layout** window is displayed.



Design Layout

Define the plate layout using the **Well Assignment** dialog box on the right side of the screen.



In the **Identifiers** group box, select **BL (Blank)**.

In the **Experimental** group box the number **1** remains.

In the **Replicates** group box, **All** is selected automatically.

Click well **A1**, which is then marked with a red border.

Click **Fill selection** and the well is labeled with the selected identifier type.

Note
A single well can also be filled by double-clicking it.

Now choose the following settings in the **Well Assignment** dialog box:

In the **Identifiers** group box, select **NC** (Negative Control).

In the **Experimental** group box the number **1** remains.

All in the **Replicates** group box is selected automatically.

Starting at well **B1** click and drag the mouse to **C1**. The wells **B1** to **C1** are then marked with a red border.

Click **Fill selection** and the wells are labeled with the selected identifier type.

Calibrators (standards) must be assigned to wells **D1** to **G2**. Select the following settings in the **Well Assignment** dialog box:

In the **Identifiers** group box, select **ST (Standard)**.

In the **Experimental** group box the number **1** remains.

In the **Replicates** group box, choose between **Fix number** and **All**.

Fix number:

Only enabled for standards and samples where IDs can be used.
If this **Fix number** button is active a number can be entered in the corresponding text field. This number defines how many replicates are intended for this method. In the selected wells, the entered number of replicates for every ID is created. Therefore the number of selected wells must be a multiple of the entered number of replicates.

All:

All selected wells are defined as replicates. If an existing ID number for the samples and standards is chosen, the selected wells are then added as replicates to the existing replicates. With all other identifier types the selected wells are added as replicates to the existing replicates.

Two arrow buttons  define the direction of the replicate and ID number sequence (horizontal or vertical).

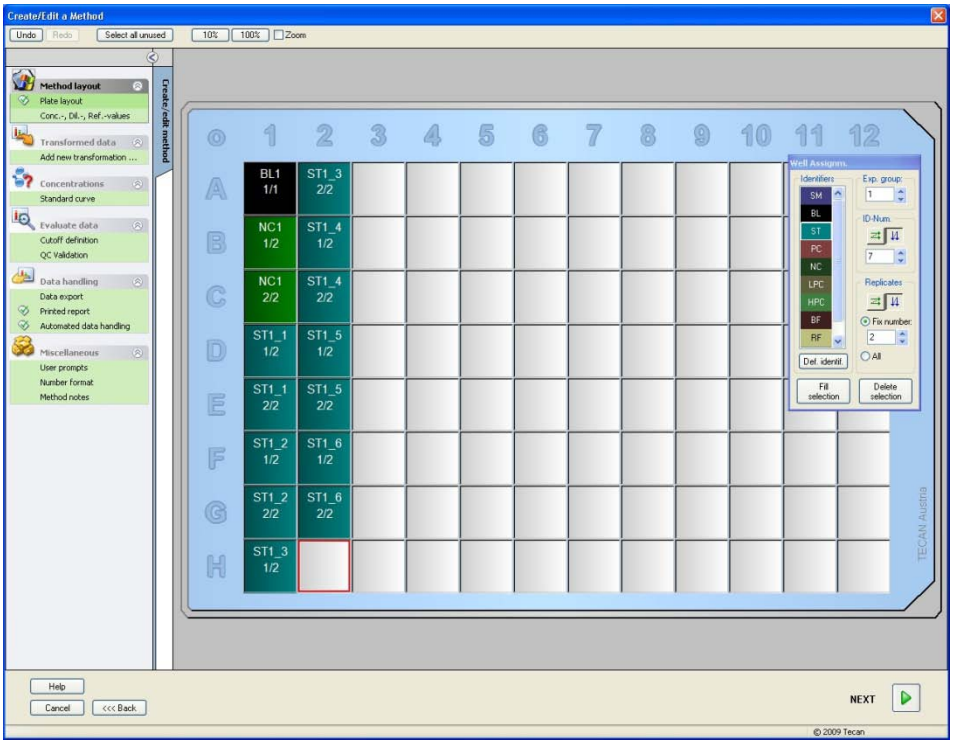
In this example select **Fix Number** and **2**.

In the **ID-Number** box and in the **Replicates** group box select the **vertical arrows**.

Then select the wells D1 to G2 and click **Fill selection**.

Note
Select the wells as follows: Starting at well D1 click and drag the mouse over the required wells to H1. Then hold down the control (Ctrl) key and drag the mouse over the required wells from A2 to G2.

The **Plate Layout** appears as follows:



Click **Select all unused** from the toolbar to select all empty wells on the plate. Then hold down the control (Ctrl) key and click the well **H12**, so that it remains blank and unmarked.

In the **Well Assignment** dialog box select **SM (Sample)** under *Identifiers*.

In the **Experimental** group box the number **1** remains.

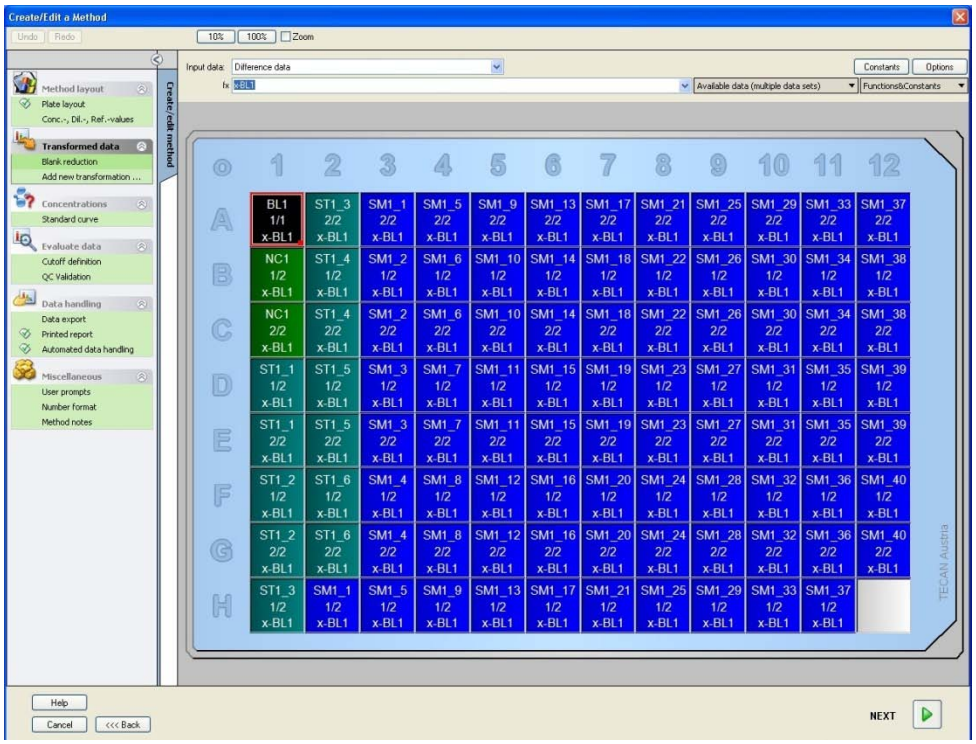
In the **Replicates** group box choose **Fix number** and **2**.

In the **ID-Number** box leave 1 and in the **Replicates** group box select the **vertical arrows**. Click then **Fill selection**. The layout definition procedure is complete.

Transformations

In the control bar on the left of the window select the next option, **Add new transformation** from the **Transformed data** item, to define blank reduction.

A dialog box appears asking you if you want to define a blank reduction. Click **Yes**. The following window appears:



Difference Data is selected automatically in the **Input data** box. If you have confirmed the definition of a blank reduction before, the software automatically names it **Blank reduction** (see transformed data in the control bar).

In the **Formula** box automatically appears **x-BL1** for this blank reduction, where x refers to the current input data value in a well and BL1 is the mean value of the blank wells of experimental group 1.

For further details and explanations concerning the definition and assignment of transformations, refer to the LT-com Instructions for Use.

In each well the following information appears (example well A5):

SM1_9	Sample, experimental group number 1, sample ID number 9.
2/2	Number of replicate is 2, total number of replicates is 2.
x-BL1 or 1	Assigned transformation x-BL1 (when Transformation is selected) or Dilution Factor value of 1 (when Conc., Dil,. Ref.-values is selected).

Concentration / Dilution / Reference Value Definition

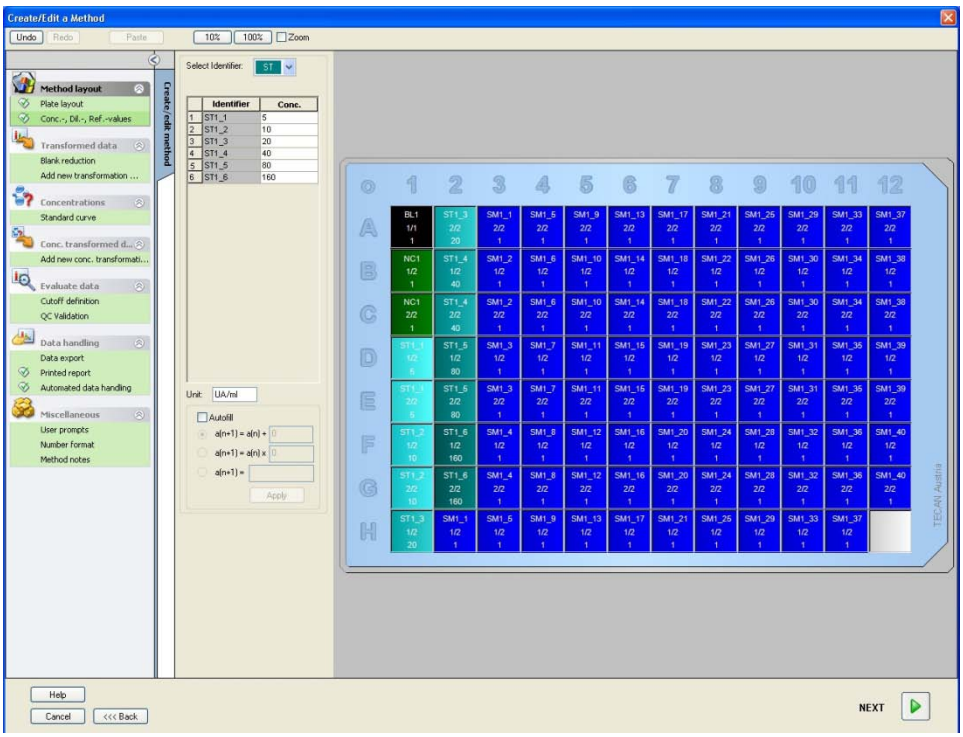
In the control bar select **Conc., Dil., Ref.-value**s from the **Method layout** item to define the respective values as described in the test kit.

Calibrator 1	5 UA/ml
Calibrator 2	10 UA/ml
Calibrator 3	20 UA/ml
Calibrator 4	40 UA/ml
Calibrator 5	80 UA/ml
Calibrator 6	160 UA/ml

Make sure **ST** is selected in the **Select Identifier** list.

In the **Identifier** list, a list of the standards from the Exp. Group 1 appears. In the corresponding **Concentration** box of **ST1_1** type the number **5** and in the **Unit** box, type UA/ml. In the corresponding **Concentration** box of **ST1_2** type the number **10**. The unit only needs to be defined once and is valid for all standards. Type the values for the ST1_3 to ST1_6 in the same way.

The screen showing the plate layout and the concentration is displayed:



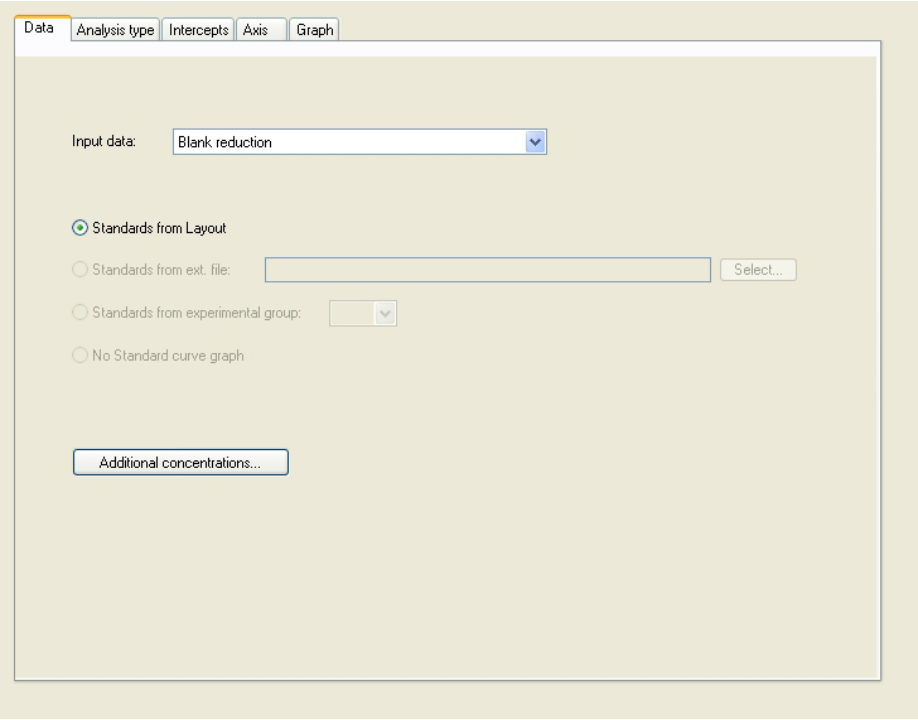
Standard Curve

In the control bar click **Standard curve** from the **Concentrations** item to define the appropriate standard curve.

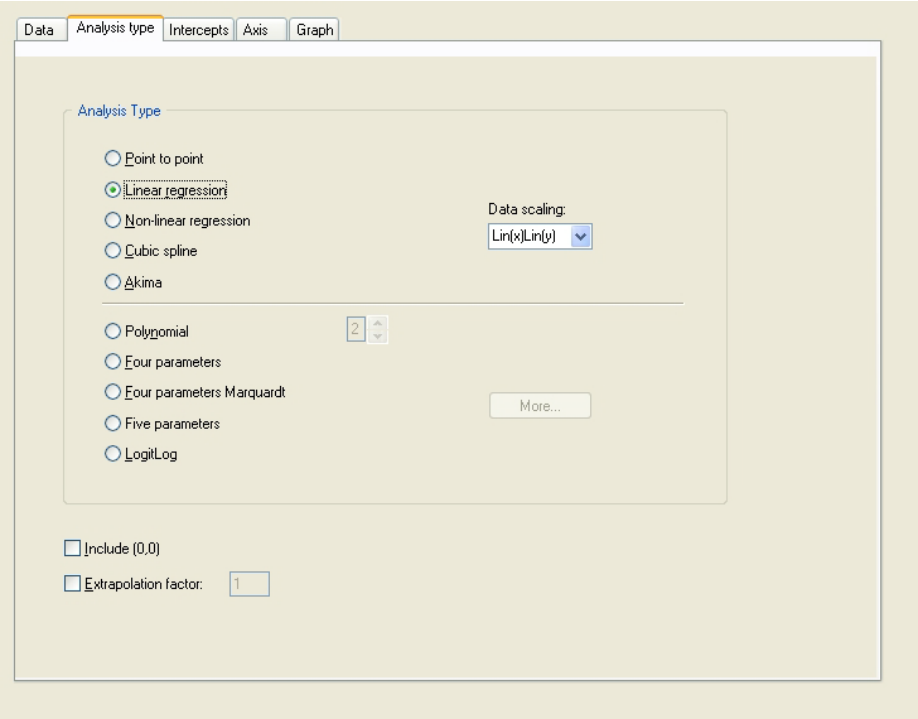
The following is in the test kit description:

After the blank correction, the optical densities (OD 492 - OD 620) are plotted versus the concentration. The regression line that goes through these points is the standard curve.

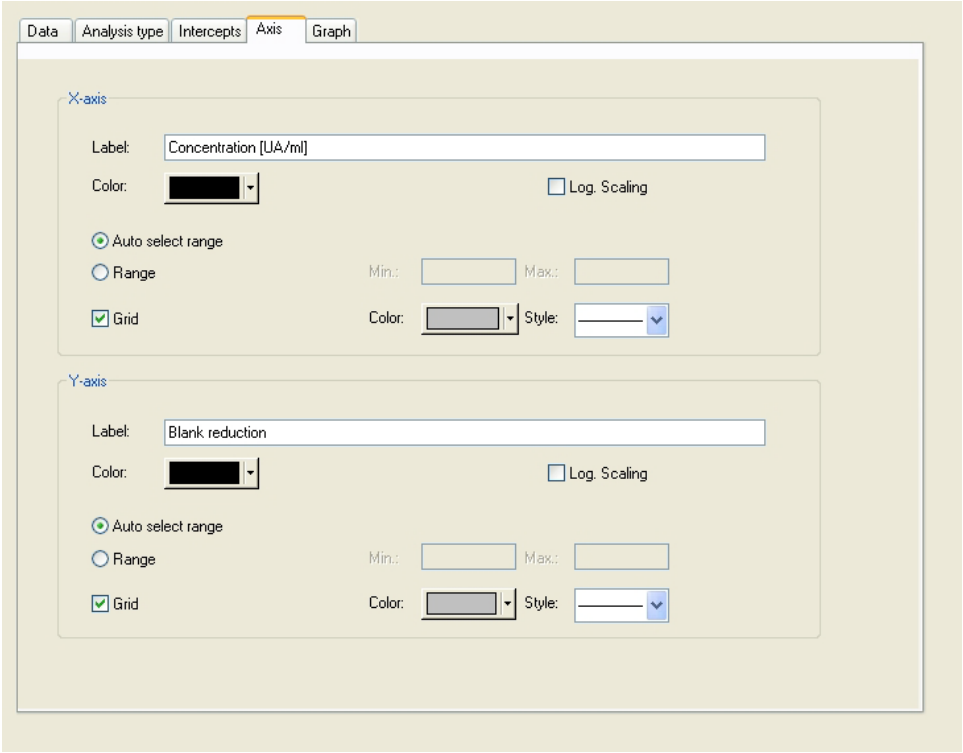
On the **Data** tab**,** select **Blank reduction** as input data.



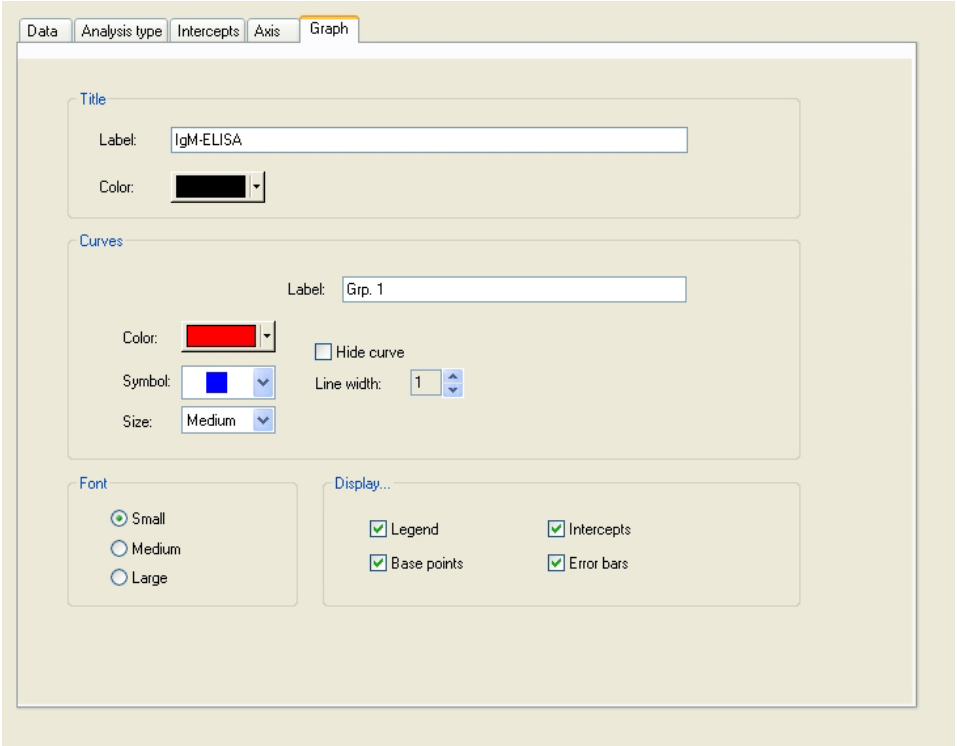
On the **Analysis type** tab, select Linear regression.



On the **Axis** tab, define the labeling and the scaling of the axis as shown below:



On the **Graph** tab, define the graph title, curves, font and graph display.



Define Cutoffs

In the control bar select **Cutoff definition** from the **Evaluate data** item to define the limits for the qualitative evaluation.

The test kit description contains the following instructions:

Interpretation of the test results:

IgM < 18 UA/ml	Negative
18 UA/ml ≤ IgM < 22 UA/ml	Intermediate
IgM ≥ 22 UA/ml	Positive

Use the following procedure to define the appropriate cutoffs:

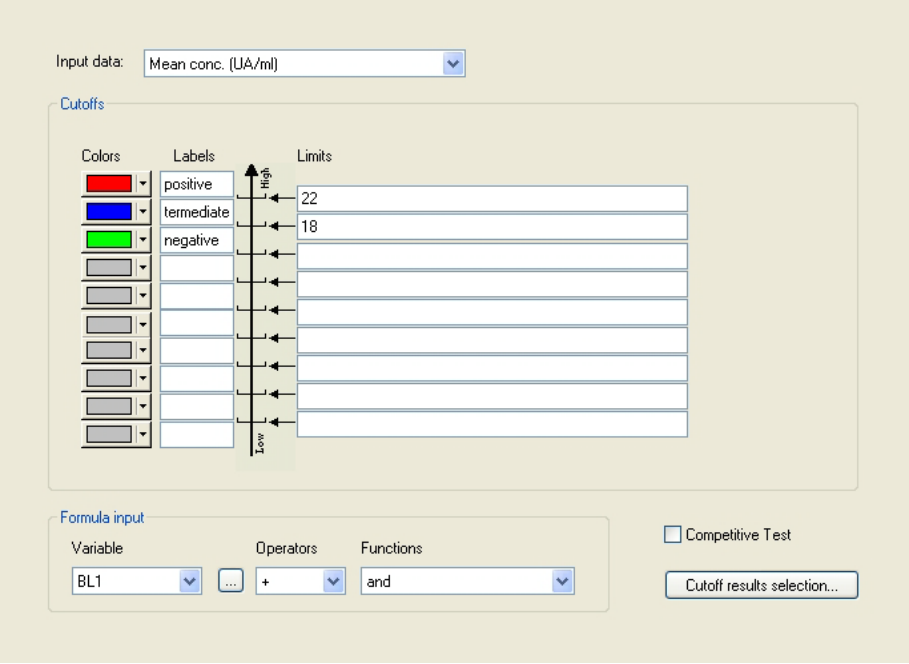
In the Input data box, select **Mean conc. (UA/ml)**.

The **Cutoffs** table represents a scale indicating the high and the low end for the **Limits** and **Labels**. In **Limits**, type 22 as the first (higher) limit and 18 as the second (lower) limit.

In **Labels**, enter the test interpretation (**Positive**, **Intermediate** and **Negative**) into the individual boxes. Use the drop-down color palette to assign a color:

- Positive – Red
- Intermediate – Blue
- Negative – Green

The screen contains the following:



Click **Cutoff results selection** to select the identifier types for which the cutoff results must be shown.

Define QC Validations

In the control bar, click **QC Validations** from the **Evaluate data** item. Validation criteria for the test must be defined, so that the validity of the test results is guaranteed.

In this example the following requirement must be fulfilled:

The calculated IgM-concentration of both negative controls must be under 8 UA/ml.

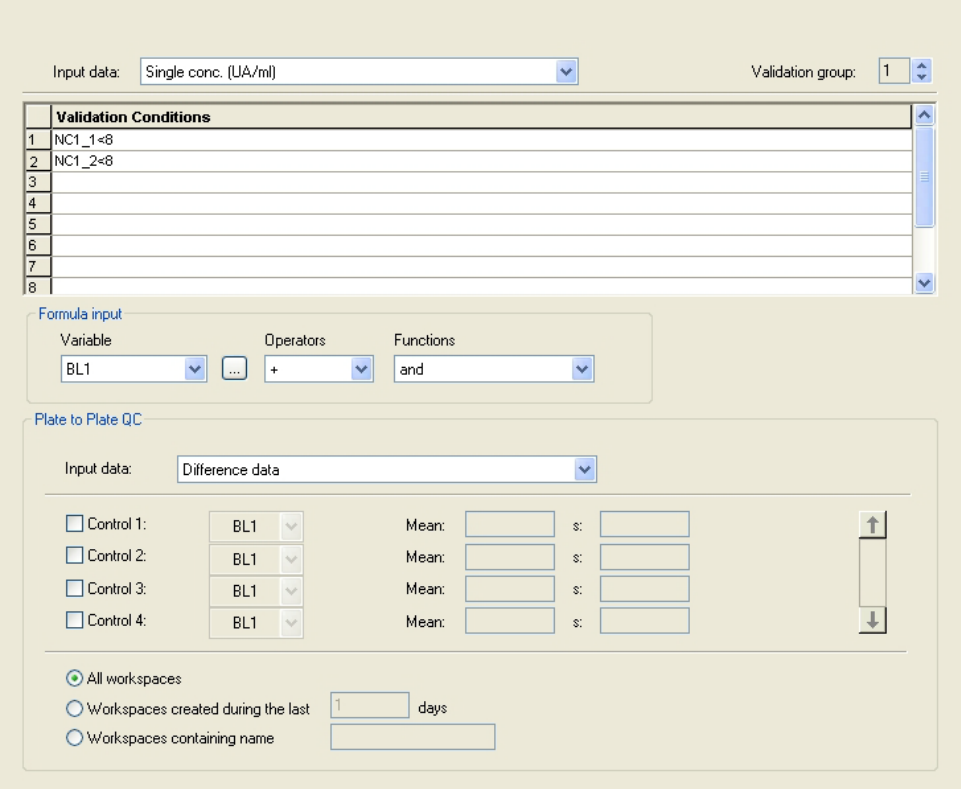
In the Input box, select **Single conc. (UA/ml)**.

In the first row, type **NC1_1<8**

Note
NC1_1 means Negative control of experimental group 1, replicate 1.

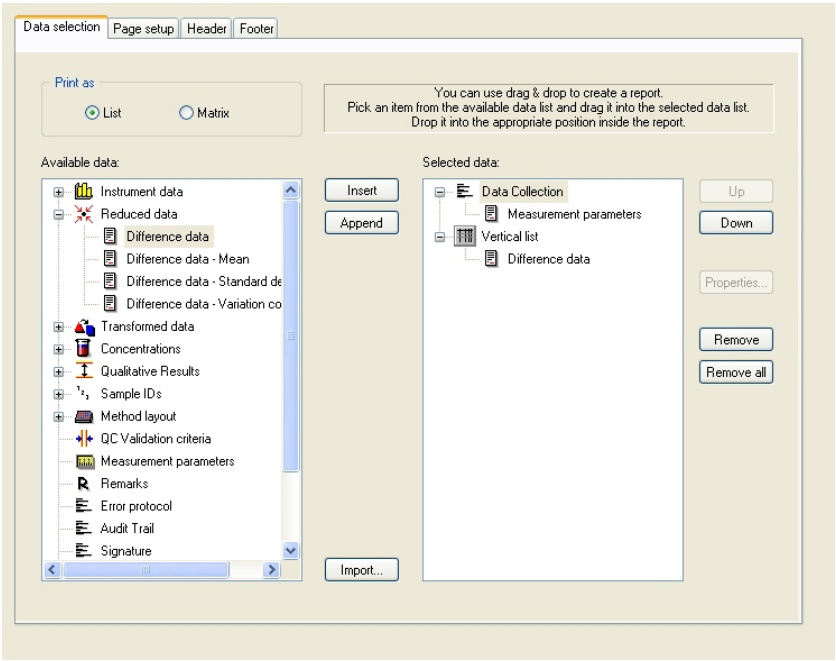
In the second row, type **NC1_2<8**.

The **QC Validations** dialog box is now displayed as follows:



6.2.3 Organize Printed Report

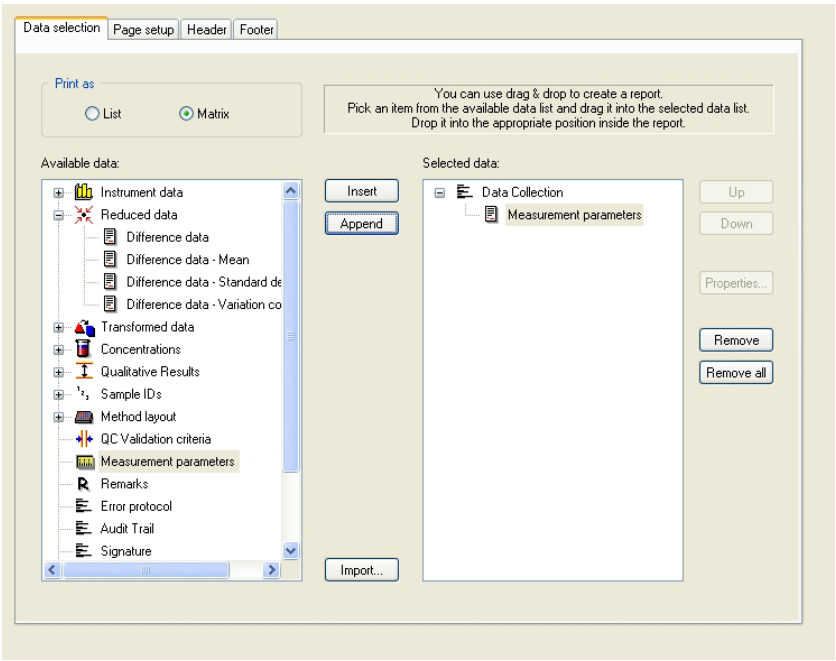
In the control bar, click **Printed repor**t from the **Data handling** item. The following screen is displayed:



On the **Data selection** tab, all available report data is contained in the **Available data** box. Using the **Insert and Append** buttons, data can be transferred into the **Selected data** box. Data can also be transferred using drag-and-drop.
In the **Print as** box, choose between printing the data as a matrix or as a list with a special orientation.

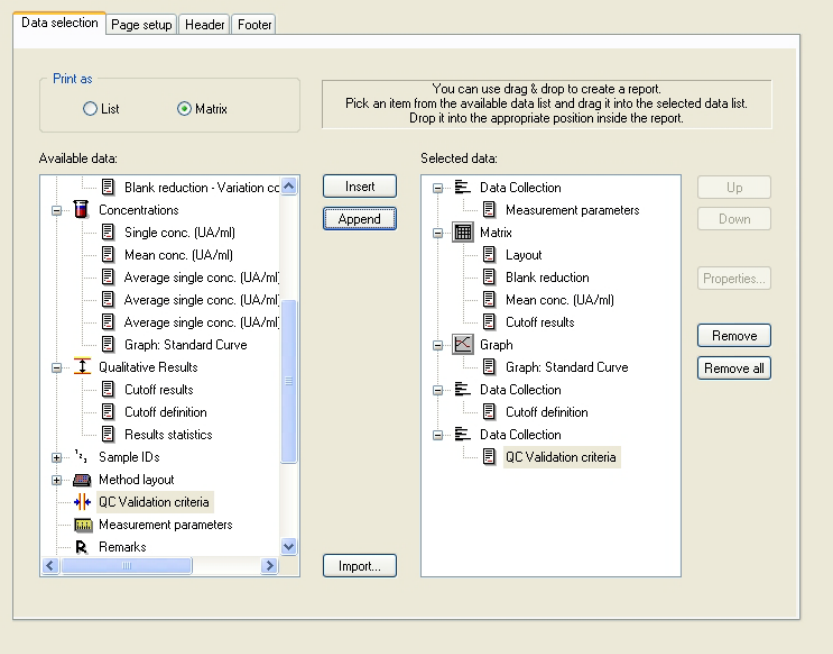
In this example a report containing the measurement parameters, plate layout, blanked values, standard curve, IgM-concentrations, cutoff definition, qualitative results of the samples and validations should be created.

Before creating the report, the default **Vertical list/Difference data** must be removed from the **Selected data** box. Only **Measurement parameters** remain in the **Selected data** box. **Print as List** must be changed to **Print as Matrix**.



Select **Method layout/Layout** in the **Available data** box and attach it as a matrix to the report by clicking **Append**. Then insert **Blank reduction**, **Mean conc. (UA/ml)** and **Cutoff results** into the matrix by selecting the corresponding items and clicking **Insert**.

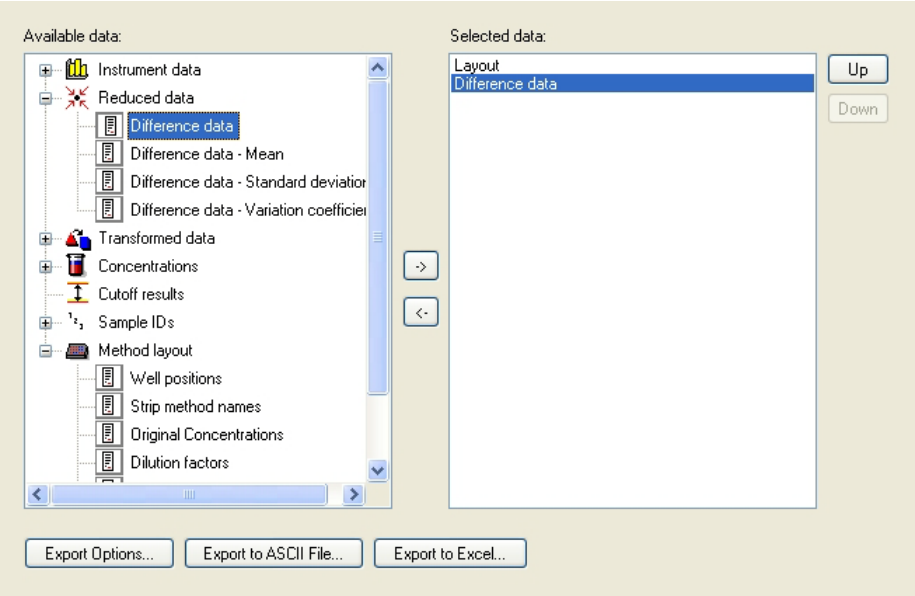
Append Graph: Standard curve, Cutoff definition and **QC Validation criteria** to the selected data. The data setup part of the report definition procedure is complete; the **Printed Report** dialog box looks like this:



On the **Header** and **Footer** tabs, define the layout of the header and the footer of the report (see LT-com Instructions for Use for further details).

Data Export

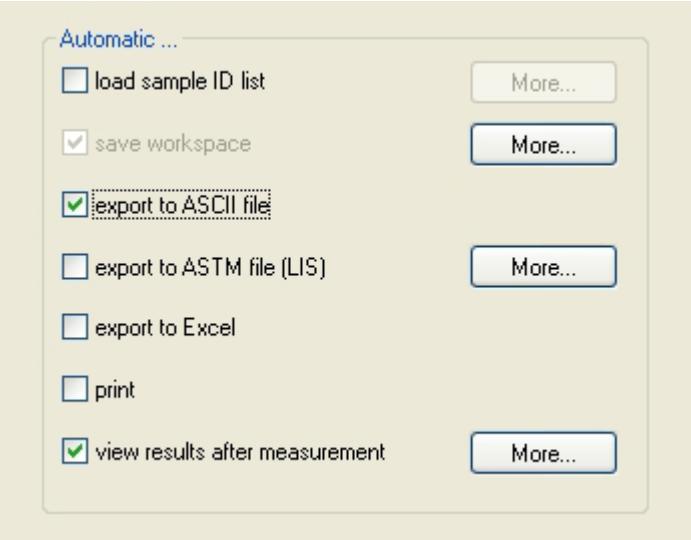
In the control bar, select **Data export** from the **Data handling** item. In this example, the layout and cutoff results should be stored as ASCII file. Select **Layout** and **Cutoff** results from the **Available data** window; click the → arrow to insert them into the **Selected data** window. The screen displays the following information:



Note
Exported data should always contain the Layout or Sample ID List.

Automated Data Handling

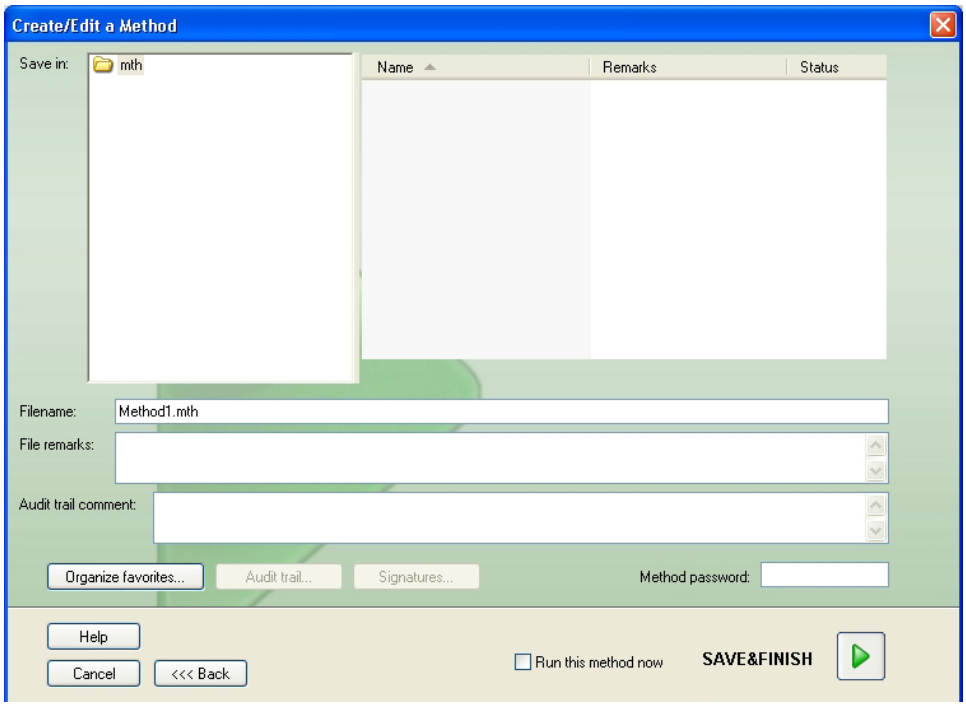
In the control bar, select **Automated data handling** from the **Data handling** item.



Select **export to ASCII file**, and **view results after measurements**. In LT-com**, save workspace** is selected by default and cannot be modified.

Save the Method

Click **Next** to open the **Save as** window. Enter the method filename and complete any other field if appropriate.

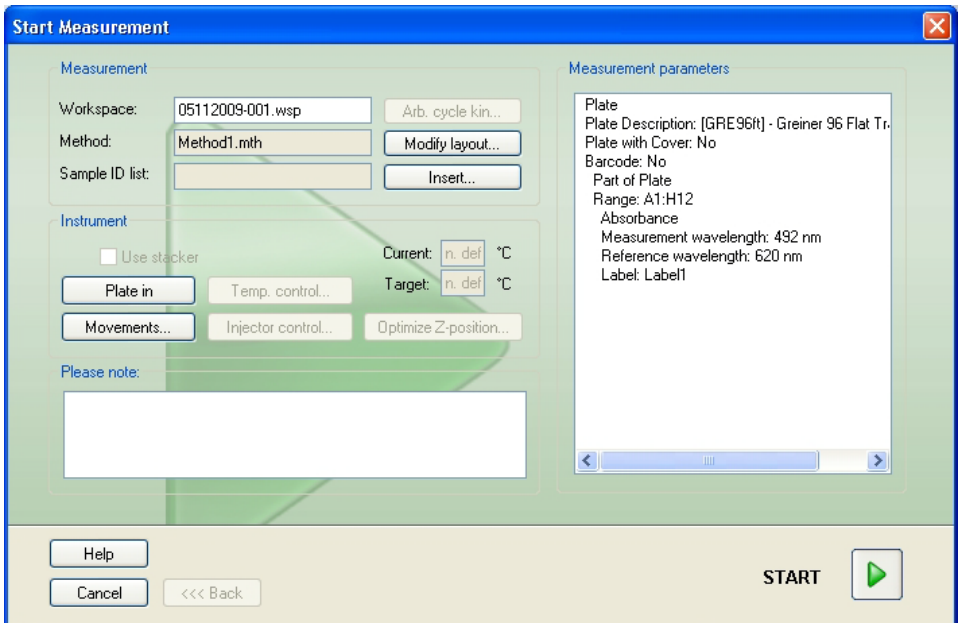


Filename text field	A filename must be entered. A default filename is suggested automatically, but can be changed.
File remarks text field	Comments entered here will be saved and displayed with the filename.
Audit trail comment text field	Comments entered here will be stored in the audit trail.
Organize Favorites button	The **Organize Favorites** dialog box appears.
Method password text field	Enter a method password to protect the method.
Run this method now check box	The method will be run immediately after clicking Save & Finish.

For further details, please refer to the LT-com Instruction for Use.

6.2.4 Run the Method

If **Run this method now** is selected in the **Save as** dialog box of the **Create/edit a method wizard**, the **Start Measurement Wizard/Start Measurement** dialog box will appear after **Save** is clicked. In the **Start Measurement** dialog a default workspace name is assigned and can be altered by the user if desired.



Click **Start** to start the measurement. A workspace will be created automatically, which contains all previously entered information and will collect all measurement values. While the measurement is being executed, a measurement status dialog box appears indicating the progress of the measurement.

After the measurement is completed, the **Results** dialog box appears, in which all the results and calculations can be viewed. Error messages can occur when performing a measurement without the according liquids (e.g. standards).

6.2.5 Evaluate the Result

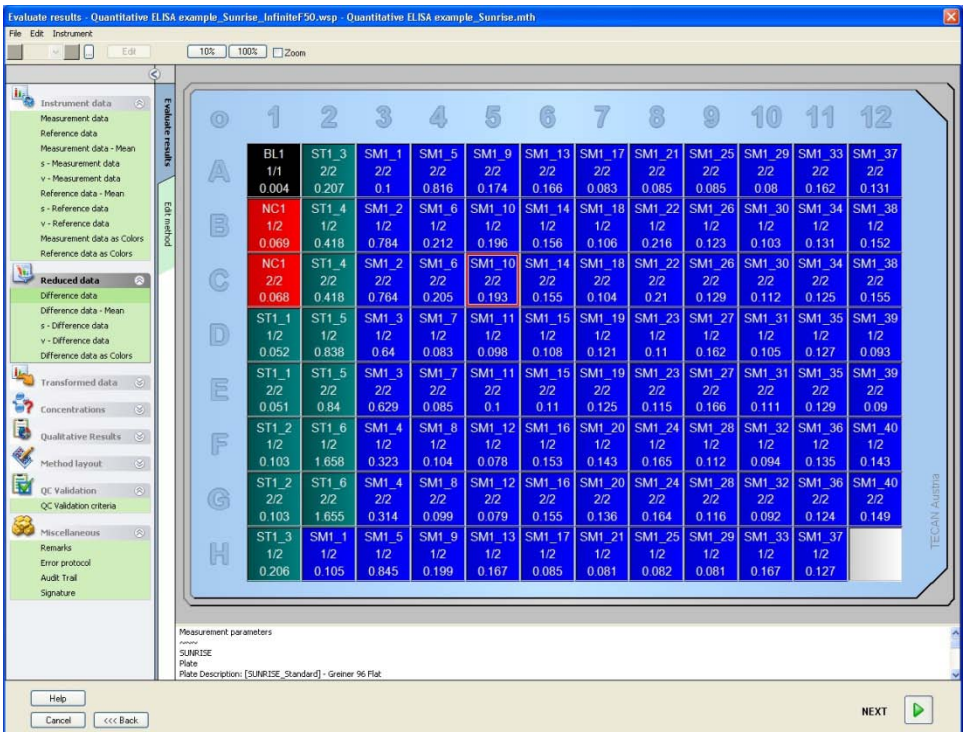
Select **Evaluate results** to view and evaluate raw data. The evaluation parameters can be viewed and data can be re-evaluated.

This section guides you through the **Evaluate Results wizard** using an example workspace file automatically installed upon installation of LT-com.

In the *Wizard List* dialog box, click **Evaluate results**.

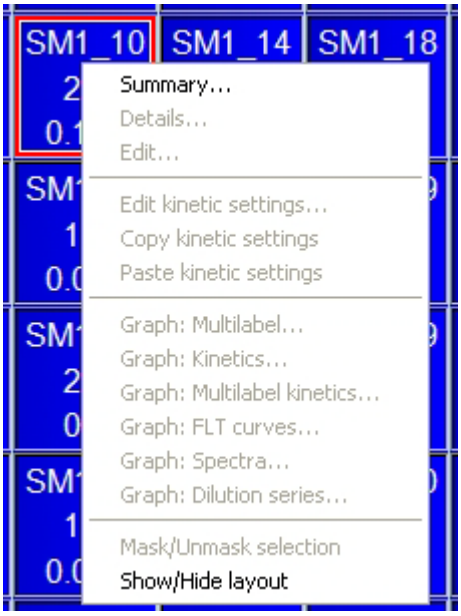
Click **Next** on the **Welcome** page of the **Evaluate Results wizard** and the **Select a file** dialog box appears.

Select the workspace **Quantitative ELISA example_LT-4500.mth** from the file list and click **Make your selection**. Calculations are executed and the following plate layout window is displayed:

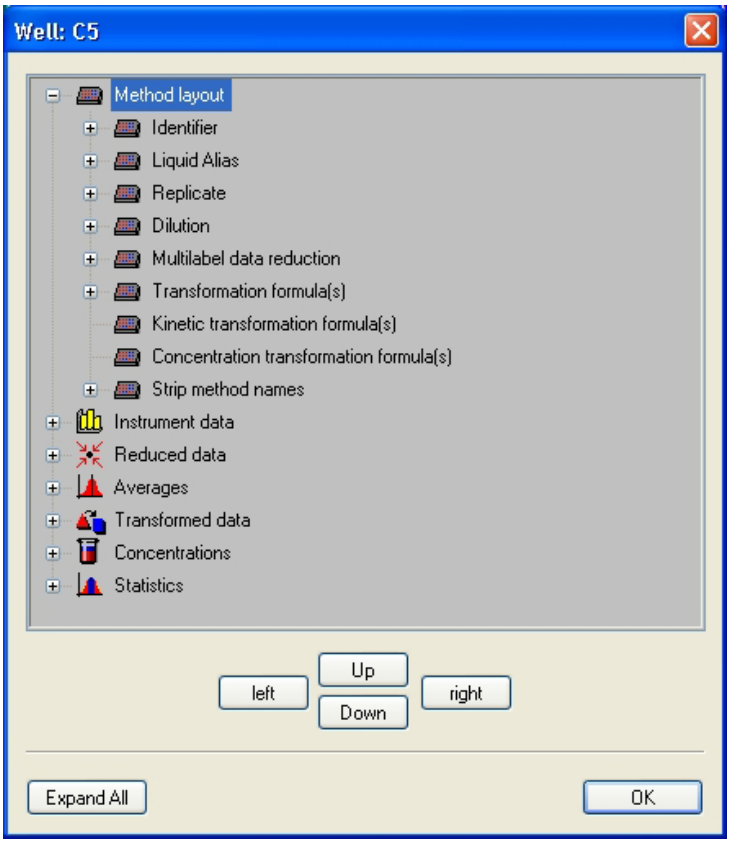


In each single well the calculated value is displayed. Depending on the selected item in the control bar, the plate layout window changes correspondingly. Parameters and settings can be changed using the items in the control bar. If the method is to be modified, click on the **Edit method** tab.

Click in the well with the right mouse key and the context-sensitive menu appears:



Selecting **Summary** the following window is displayed providing detailed information of the definition and the settings of the chosen well:



Click **Next** in the plate layout window and the **Save as** dialog box appears, where you can enter a file name and remarks. Click the small **Save** button on the left of the window to save the file; you can continue working on the method or workspace. Click the **Finish** button on the right side at the bottom of the screen to save the file and to close the wizard. The program goes back to the wizard list.

6.2.6 *Summary of Definition of Quantitative ELISA in LT-com*

1. Subtract Blank value

Definitions in LT-com

Click on **Add new transformation** in the control bar and a window appears, asking if you want to define a **Blank reduction**. Click **Yes** and the **Blank reduction** formula is assigned automatically to all wells.

2. Define Concentrations

Definitions in LT-com (Control bar – Method layout/ Conc.-, Dil.-, Ref.-values)

Selected identifier: ST

Unit: UA/ml

ST1_1	5	(ST1_1	..Standard 1 first experimental group)
ST1_2	10	(ST1_2	..Standard 2 first experimental group)
ST1_3	20	(ST1_3	..Standard 3 first experimental group)
ST1_4	40	(ST1_4	..Standard 4 first experimental group)
ST1_5	80	(ST1_5	..Standard 5 first experimental group)
ST1_6	160	(ST1_6	..Standard 6 first experimental group)

3. Define Standard Curve

Definitions in LT-com (Control bar – Concentrations/ Standard curve)

Input data	blank reduction
Analysis type	linear regression
X-axis	linear
Y-axis	linear

4. Define Cutoffs

Definitions in LT-com (Control bar – Evaluate data/ Cutoff definition)

Input data: Mean conc. (UA/ml)

Limits: 22

18

Positive ≥ 22 > intermediate ≥ 18 > negative

Non-competitive test

5. QC Validation

Definitions in LT-com (Control bar – Evaluate data/QC validation):

Input data: Single conc. (UA/ml)

Validation condition 1 NC1_1<8

Validation condition 2 NC1_2<8

NC1_1 ..Negative Control first replicate first experimental group

NC1_2 ..Negative Control second replicate first experimental group

7. Cleaning, Maintenance, and Disposal

7.1 Introduction

This chapter contains the following procedures:

- Clean the instrument
- Disinfect the instrument
- Maintain the instrument
- Insert or replace filters in the filter wheel
- Disposal instructions

WARNING

BEFORE DOING ANY CLEANING OR MAINTENANCE REMOVE THE MICROPLATE.

WARNING

PRIOR TO CLEANING AND DISINFECTING DISCONNECT THE INSTRUMENT FROM THE EXTERNAL POWER SUPPLY.

CAUTION

DO NOT MOVE THE PLATE TRANSPORT MANUALLY UNLESS THE INSTRUMENT IS SWITCHED OFF.

7.2 Cleaning the Instrument

WARNING

THE CLEANING PROCEDURE SHOULD BE PERFORMED IN A WELL-VENTILATED ROOM BY AUTHORIZED TRAINED PERSONNEL WEARING DISPOSABLE GLOVES AND PROTECTIVE GLASSES AND CLOTHING.

Clean the housing of the device and the plate transport only with a dry or moist cloth. If very dirty, clean it with a cloth moistened with a maximum of 70 % ethanol or mild detergent, Microcide SQ, or Decon 90. Wipe dry with a paper towel cloth.

If any liquid is spilled on the instrument, it should be immediately removed to prevent liquid running into the optical system causing loss of performance or the error message **Lamp Low**.

7.3 Instrument Disinfection

WARNING

IF LIQUID SPILLED ON THE PLATE TRANSPORT IS POTENTIALLY INFECTIOUS IT SHOULD BE DISINFECTED ACCORDING TO THE RELEVANT NATIONAL LAWS AND REGULATIONS.

All parts of the instrument that came into contact with biological samples, patient samples, positive control samples, or hazardous material must be treated as potentially infectious areas.

WARNING

THE DISINFECTION PROCEDURE AND THE DISINFECTANTS SHOULD CONFORM TO THE RELEVANT NATIONAL LAWS AND REGULATIONS.

WARNING

IT IS VERY IMPORTANT THAT THE INSTRUMENT IS THOROUGHLY DISINFECTED BEFORE IT IS REMOVED FROM THE LABORATORY OR BEFORE ANY SERVICE IS PERFORMED ON IT.

Before the instrument is returned to the local sales representative or to a service center, all surfaces and the plate transport must be disinfected and a safety certificate must be completed by the operating authority. If a safety certificate is not supplied, the instrument may not be accepted by the local sales representative or service center or custom authorities may hold it.

7.3.1 Disinfection Solutions

The outer surfaces and the plate transport of the instrument should be disinfected using a disinfectant such as:

- Microcide SQ
- Decon 90
- 70 % Ethanol

WARNING

RISK OF FIRE AND EXPLOSION!

ALCOHOLS, SUCH AS ETHANOL OR ISOPROPANOL, ARE FLAMMABLE AND WHEN IMPROPERLY HANDLED CAN LEAD TO EXPLOSIONS AND/OR FIRE. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

CAUTION
NEVER USE ACETONE AS IT WILL DAMAGE THE COVERS.

7.3.2 Disinfection Procedure

If the laboratory has no specific disinfection procedure, the following procedure should be used to disinfect the outer surfaces and the plate transport of the instrument.

WARNING

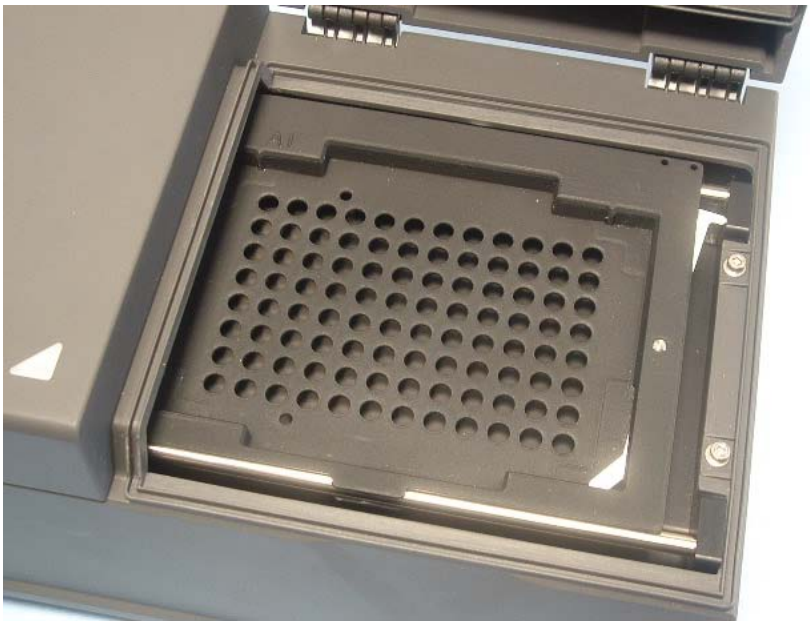
THE DISINFECTION PROCEDURE SHOULD BE PERFORMED IN A WELL-VENTILATED ROOM BY AUTHORIZED TRAINED PERSONNEL WEARING DISPOSABLE GLOVES AND PROTECTIVE GLASSES AND CLOTHING.

CAUTION
THE SURFACE DISINFECTANT CAN NEGATIVELY INFLUENCE THE PERFORMANCE OF YOUR INSTRUMENT, IF IT IS APPLIED OR ACCIDENTALLY GETS INSIDE THE INSTRUMENT.

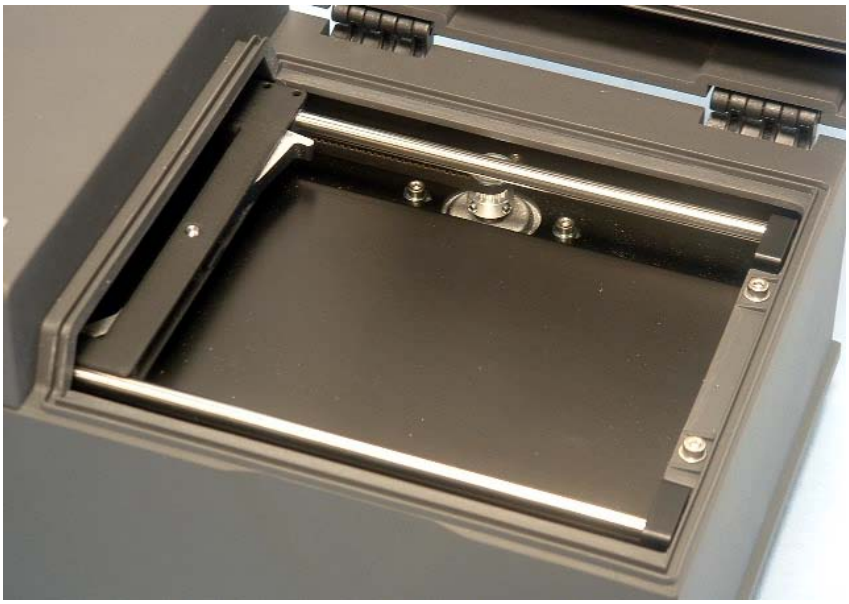
WARNING

PRIOR TO DISINFECTION DISCONNECT THE INSTRUMENT FROM THE MAIN POWER SUPPLY TO AVOID ANY RISK OF FIRE OR EXPLOSION.

1. Wear protective gloves, protective glasses, and protective clothing.
2. Prepare a suitable container for all disposables used during the disinfection procedure.
3. Disconnect the instrument from the software and switch the instrument off.
4. Carefully move the plate transport out of the instrument.



5. Carefully apply the disinfectant solution on the plate transport according to the manufacturer's Instructions for Use.
   Do not use too much disinfectant to prevent the solution flowing into the instrument or soiling the lenses when moving the plate transport into the device.
6. After the required contact time (according to the manufacturer's Instructions for Use) wipe the plate transport using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the disinfectant.
7. Carefully move the plate transport into the instrument.



8. Carefully apply the disinfectant on the base plate of the plate transport.
9. After the required contact time wipe the base plate of the plate transport using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the disinfectant

10. Carefully apply the disinfectant solution on all outer surfaces of the instrument.
11. After the required contact time wipe the instrument using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the disinfectant.
12. Wipe dry the outer surface of the instrument with a soft paper towel.
13. Repeat the disinfection procedure on any accessories which are being moved or returned.
14. Dispose of the container with the disposables according to the relevant national laws and regulations.
15. Disinfect your hands and clean them with a mild detergent.

When sending the instrument back to the local sales representative/service center please continue with the following steps:

16. Pack the instrument and its accessories.
17. Complete the safety certificate (see below) and attach it to the outside of the box so that it is clearly visible.

7.3.3 Safety Certificate

To ensure the safety and health of personnel, our customers are kindly asked to complete a **Safety Certificate** (which was delivered with the instrument) and attach one copy to the top of the container in which the instrument is returned (visible from the outside of the shipping container!) and another copy to the shipping documents before shipping it to the service center for service or repair.

The instrument must be disinfected at the operating authority's site before shipping (see 7.3.2 Disinfection Procedure).

The disinfection procedure must be performed in a well-ventilated room by authorized and trained personnel wearing disposable powder-free gloves, protective glasses and protective clothing.

The disinfection procedure should be performed according to national, regional, and local regulations.

If a Safety Certificate is not supplied, the instrument may not be accepted by the servicing center.

Your local supplier can send you a new copy of the Safety Certificate, if required.

7.4 Preventive Maintenance Plan for LT-4500

The following preventive maintenance procedures are recommended.

7.4.1 Monthly

Clean the housing and the plate transport with a mild detergent at least once per month; more often when necessary.

CAUTION
NEVER USE ACETONE AS IT WILL DAMAGE THE COVERS.

7.4.2 Every 4 Years

It is recommended to replace the filters every 4 years.

7.5 Filter Replacement and Installation

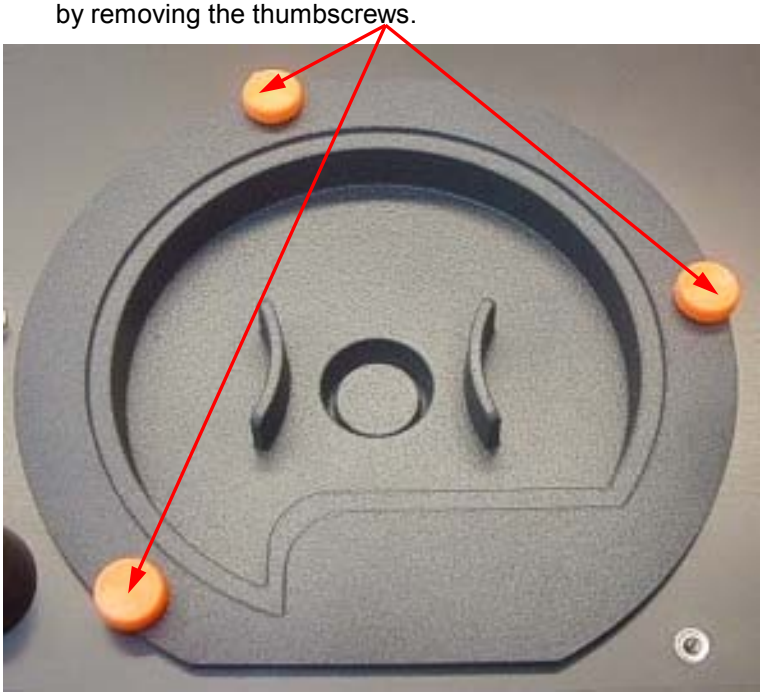
The LT-4500 must be connected to LT-com in order to do a software guided filter insertion or replacement. If the connection is lost during the procedure, due to accidental interruption of the connection between the instrument to the computer, LT-com has to be terminated and the instrument has to be switched off. In this case, continue the procedure as stated below. When finished, reestablish the connection by restarting the device and LT-com and define the newly inserted filters.

CAUTION
WHEN HANDLING THE FILTERS, BE CAREFUL THAT THEY DO NOT BECOME SCRATCHED OR SOILED WITH FINGERPRINTS OR DUST.

7.5.1 Filter Switching Procedure

The filters of the standard filter wheel can be replaced or supplemented using the following procedure:

1. In the Wizard list window, click **Miscellaneous**.
2. Click **Instrument control**
3. Click **Define filter slides**
4. Click **Filter switching** to start the procedure.
5. Remove any microplate from the plate transport!
6. Tilt the instrument carefully backwards until it lies on the back side with the bottom facing towards you.
7. Remove the cover plate from the bottom of the instrument by removing the thumbscrews.

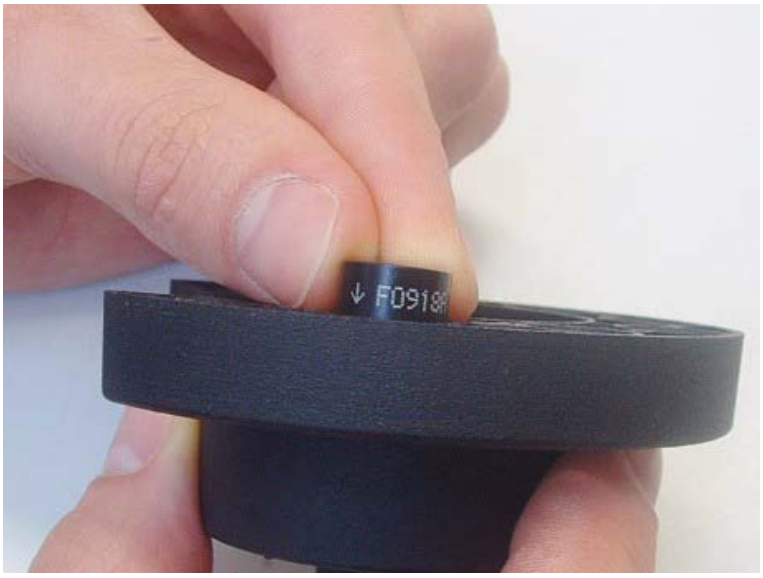


8. Remove the magnetically fixed filter wheel by carefully pulling it out of the instrument.

9. Place the filter wheel on a clean flat surface.

10. When replacing a filter, use the filter assembly tool to remove the filter from the filter slot.
    Please contact your local supplier for the filter assembly tool and available filters.

11. Align the filter assembly tool with the notch of the stop-ring. Turn the tool and remove the stop-ring by pulling it out of the filter slot.
12. Turn the filter wheel over so that the filter slides out of the slot. Do not use the filter assembly tool to push filters out of the filter slot, as the filter could get scratched.
13. A new filter must be inserted into the filter slot in the correct direction taking care not to scratch the filter or get fingerprints on it.



Note
Make sure that the filter is inserted correctly.

14. Place the stop-ring on the end of the filter assembly tool and turn it so it cannot slip off.

15. Using the filter assembly tool, push the stop-ring into the filter slot and press firmly into place.
16. Rotate the tool until the notch in the stop-ring is aligned with the end of the filter assembly tool and remove the tool.

17. Place the filter wheel back into the filter wheel slot and push it deeper until it is secured magnetically.
18. Reattach the cover plate back onto the bottom of the instrument with the three orange screws.
19. Bring the device back into an upward position.
20. Click **OK** to finalize the procedure and initialize the filter wheel.
21. Define the newly inserted filter (see next chapter for detailed procedure).

7.5.2 Defining Filters

In the **Filter Definition** dialog box assign appropriate wavelengths to replaced filters by entering the new wavelengths in the corresponding positions.

If a filter has been inserted into a new filter position, activate the appropriate filter position by selecting the check box and entering the appropriate wavelength.

By clicking **Save**, the filter definitions will be saved and the filters will be initialized.

Once the filters have been initialized, the instrument is ready for measurements.

Note
Be careful not to mix up the filter positions and filter wavelengths as this will lead to wrong measurement data.

7.6 Disposal

7.6.1 Introduction

Follow laboratory procedures for biohazardous waste disposal according to national and local regulations.

This chapter provides instructions on how to lawfully dispose of waste material accumulating in connection with the LT-4500.

CAUTION
OBSERVE ALL FEDERAL, STATE, AND LOCAL ENVIRONMENTAL REGULATIONS.

7.6.2 Disposal of Packing Material

The packing material consists of recyclable material. If you do not intend to keep it for future use, e.g. for transport and storage purposes, please dispose of the packing material according to local regulations.

7.6.3 Disposal of Operating Material

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESS RUN ON THE LT-4500 ABSORBANCE READER.

TREAT THE USED MICROPLATE, OTHER DISPOSABLES, AND ALL SUBSTANCES USED IN ACCORDANCE WITH GOOD LABORATORY PRACTICE GUIDELINES.

INQUIRE ABOUT APPROPRIATE COLLECTING POINTS AND APPROVED METHODS OF DISPOSAL IN YOUR COUNTRY, STATE, OR REGION.

7.6.4 Disposal of the Instrument

If you have any questions concerning the disposal of the device, please contact your local supplier.

Pollution degree	2 (IEC/EN 61010-1)
Method of disposal	Contaminated waste

ATTENTION

DIRECTIVE 2002/96/EC ON WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE)

NEGATIVE ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE TREATMENT OF WASTE.

- **DO NOT TREAT ELECTRICAL AND ELECTRONIC EQUIPMENT AS UNSORTED MUNICIPAL WASTE.**
- **COLLECT WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT SEPARATELY.**

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE LT-4500 MAY HAVE BEEN IN CONTACT WITH BIO-HAZARDOUS MATERIAL.

- **MAKE SURE TO TREAT THIS MATERIAL ACCORDING TO THE APPLICABLE SAFETY STANDARDS AND REGULATIONS.**
- **DECONTAMINATE ALL PARTS BEFORE DISPOSAL.**

Information for Treatment Facilities

The LT-4500 consists of the following main components:

- Poly-chlorinated biphenyl (PCB)
- Plastics
- Cable
- Metals (Iron)
- Nonferrous Metal (NFM)

The instrument is compliant with the RoHS directive 2002/95/EC on the restriction of the use of certain hazardous substances in electrical and electronic equipment.

For more information about the product, please contact:

labtech.com
Unit 2 Birch House
17 Bramble side
Bellbrook Ind Estate
Uckfield
TN22 1QQ
United Kingdom

8. Troubleshooting

8.1 Introduction

The internal microprocessor controls and checks electronic functions as well as measurements, operations and results. If the microprocessor detects a fault or an incorrect operating procedure, an error message is displayed on the computer.

8.1.1 Table of Error Messages and Troubleshooting

The following table gives a brief description of the error messages and the troubleshooting actions.

Note
If other error messages appear that are not mentioned in the table below please contact your local supplier.

Error Message	Description	Troubleshooting
System Error		
“Lid Open Error”	Lid open at start of a measurement	Close lid and start measurement again
“MTP Init Error”	MTP transport could not be initialized	Hardware problem: Electronic defect, belt broken or MTP transport mechanically blocked
“MTP lost steps abs(*steploss*) > *max_steploss*”	MTP lost steps during measurement *Steploss*: number of lost steps *max_steploss*: number of allowed steploss	Hardware problem: Electronic defect, rough-running mechanic
“Filter lost steps abs(*steploss*) > *max_steploss*”	Filter wheel lost steps during measurement. *Steploss*: number of lost steps *max_steploss*: number of allowed steploss	Hardware problem: Electronic defective, rough-running mechanic
“USB timeout”	Timeout in USB communication	System Error – report to customer support
“Lamp Low! Minimum: *minimum*, Maximum: *maximum*”	Measured light intensity did not reach the expected range between *minimum* and *maximum*	Hardware problem: Electronic defect, broken fiber
“Wavelength Not Available ! Wavelength: *wavelength* nm”	The filter with the wavelength *wavelength* could not be found in filter wheel	System error – report to customer support

Error Message	Description	Troubleshooting
“Channel Low! Channel: *channel_nr*, Minimum: *minimum*, Maximum *maximum*	Signal on channel *channel_nr* did not reach the expected area between *minimum* and *maximum*	Hardware problem: Electronic defect, broken fiber
“Invalid Wavelength! Wavelength: *wavelength*nm”	Filter wavelength is outside the range of wavelengths of the white and the blue LED	System error – report to customer support
“Lamp Overflow!” Minimum: *minimum*, Maximum: *maximum.*	Signal on ADC exceeds the expected area between *minimum* and *maximum*	Hardware problem: Electronic defect
“Value Not Set: *value -1*”	The value *value* is not set	System error – report to customer support
“Filter Init Error”	Filter transport could not be initialized	Hardware problem: Electronic defect, filter wheel transport mechanically blocked

8.1.2 Definition of ‘Overflow’

If the result of the absorbance measurement is not within the instrument specifications (> 4.0 OD) an overflow will occur and the measured OD value of the respective well will be replaced by ‘Overflow’. This is done by the controlling software and not by the instrument itself.

8.1.3 Power Failure

In case of power failure the following happens:

Power failure of the instrument, but not of the controlling computer (e.g. computer connected to uninterruptible power supply system): USB connection between instrument and computer will be lost. Error message by the controlling software is created.

Power failure of both instrument and controlling computer: the computer has to reboot. No measurement data will be available.

9. Abbreviations, Trademarks, and Symbols

9.1 Abbreviations

The following abbreviations are provided as a reference and may appear in the Instruction for Use.

A	Ampere
AC	Alternating Current
ADC	Analog Digital Converter
ANSI/SBS	American National Standards Institute/Society for Biomolecular Screening
ASCII	American Standard Code for Information Interchange
ASTM	American Society for Testing and Material
°C	Degrees Celsius
CE	Conformité Européenne
CFR	Code of Federal Regulations
cm	Centimeter
DC	Direct Current
EC	European Community
ELISA	Enzyme-linked Immunosorbent Assay
EN	European Norm
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
Hz	Hertz
IEC	International Electrotechnical Commission
ID	Identification
IFU	Instructions for Use
IQ	Installation Qualification
IVD	In vitro diagnostics
kg	Kilogram
l	Liter
LED	Light Emitting Diode
LIS	Laboratory Information System
mg	Milligram
ml	Milliliter
mm	Millimeter

MTP	Microplate
µl	Microliter
NFM	Nonferrous Metal
NIST	National Institute of Standards and Technology
nm	Nanometer
NRTL	Nationally Recognized Testing Laboratory
OD	Optical Density
OQ	Operational Qualification
PCB	Poly-chlorinated biphenyl
RF	Radio Frequency
RoHS	Restriction of the Use of Certain Hazardous Substances
SOP	Standard Operating Procedure
USB	Universal Serial Bus
UA	Arbitrary Units
TÜV	Technischer Überwachungsverein (Technical Inspection Agency)
V	Volt
VA	Volt Ampere
WEEE	Waste electrical and electronic equipment

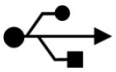
9.2 Trademarks

The following product names and any registered and unregistered trademarks mentioned in this document are used for identification purposes only and remain the exclusive property of their respective owners:

- LT-com, LT-4500 are trademarks of **labtech.com**, Uckfield, UK
- Multicheck™ is a trademark of Tecan Austria GmbH
- Windows® and Excel® are registered trademarks of Microsoft Corporation, Redmond, WA, USA
- Pentium® and Atom™ are trademarks of Intel Corporation, Santa Clara, CA, USA
- Adobe® Reader® is a registered trademark of Adobe Systems Incorporated, Seattle, WA, USA
- Microcide SQ™ is a trademark of Global Biotechnologies Inc., Portland, ME, USA
- Decon 90™ is a trademark of Decon Laboratories Ltd., Hove, East Sussex, UK

9.3 Symbols

The following symbols appear on the instrument.

	Manufactured by
	Date of manufacture
	Conformité Européenne
	Read the Instructions for Use before operating the instrument
	In vitro diagnostic medical device
	Order number
	Serial Number
	USB label
	WEEE symbol
	RoHS symbol
	TÜV NRTL
	Bio-hazardous

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