Biochrom Anthos 2020 Microplate Reader User's Manual

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1 Safety Information

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle. Please pay special attention to the specific safety information associated with these symbols.

Warning and Caution Definitions

The exclamation point symbol is an international symbol, which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

WARNING
A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION
A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

Electrical Safety
To prevent electrically related injuries and property damage, properly inspect all electrical equipment priors to use and immediately report any electrical deficiencies. Contact an Anthos service representative for any servicing of equipment requiring the removal of covers or panels.

High Voltage:

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

- Voltages dangerous to human life are present in this device. Before removing any covers disconnect the device from the power source.
- Ensure that the power cord supplied with the unit is used.
- The power cord may only be inserted in a socket outlet provided with a protective ground (earth) contact. The protective action must not be negated by use of an extension cord without a protective grounding contact.

- Do not replace fuses without first removing the main power cord. Ensure that only fuses with the required rated current and of the specified type are used for replacement. The use of makeshift fuses and the short-circuiting of fuse-holders is prohibited.
- When the apparatus is connected to the main power source, the opening of the covers or removal of components is likely to expose live parts. The device shall be disconnected from all voltage sources before it is opened for adjustment or repair
- Any adjustment or repair of the opened apparatus under voltage should be avoided, but, if necessary, it must be carried out by qualified service personnel who are aware of the hazards involved.
- Use the equipment only in the intended manner and as specified by the manufacturer, otherwise the protection provided by the equipment may be impaired.

Chemical and Biological Safety

Normal operation of the 2010 and 2020 Absorbance Detectors may involve the use of materials that are toxic, inflammable, infectious or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease. Wear protective gloves.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the 2010 and 2020 Absorbance Detectors in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.
- Wash your hands thoroughly after handling test fluids. If equipment has been in contact with hazardous substances, it must be disinfected prior to shipment in accordance with the effective provisions.

Helpful Hints:

- Make sure that the plate is inserted correctly with well A1 on the upper left position.
- Always consider that the reader is an optical instrument and is using the liquid surface and the well bottom as optical surfaces.
- Avoid foaming and make sure that no bubbles or particles in the well can disturb the measurements. Extremely strong reactions may cause substrates to precipitate.
- Visually inspect the bottom of plates before use for optical distortion and contamination.
- Use bichromatic reading for eliminating unspecific signals caused by plate material or contamination.
- When using plate frames for column/row strips or single wells (breakable strips) check that all strips and wells are pushed down completely and are level with the frame.
- Keep the bottom of the plate clean and dry. If liquid has contaminated the well bottoms, wipe off with a soft cloth or paper suited for optical surfaces. Never touch the optical surfaces with your fingers.
- Do not insert plates into the reader, which are colder than the instrument and environment. If this is required by the application, check the well bottoms for possible condensation before reading.

Moving Parts

- Do not touch the plate during movement of the plate transport (risk of injury).
- Keep the work area around the reader free of clutter.

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2 Introduction

Picture 1. Biochrom Anthos 2020 Microplate

The Biochrom Anthos 2020 is designed for all applications of endpoint and kinetic absorbency reading in 96-well microplates.

The Biochrom Anthos 2020 is equipped with a built in PC, display and keyboard and with comprehensive software functions on-board for performing measurements and data-evaluation. When operated in remote-control, it works identical to a remote instrument but in addition files stored on-board (measurement data, test-definition and evaluated result files in readable ASCII text format) can be exchanged with a desktop-PC by means of ADAP Software functions

Intended use

The Biochrom Anthos 2020 are intended for general laboratory and research use only.

3 Technical Information

General Information regarding the Absorbance Microplate Reader

1-channel single-beam absorption photometer and discontinuous wavelength feed (filter)

Metrological specification

3.1.1 Measuring system

- Light source: pre-adjusted, automatically-controlled Tungsten halogen lamp
- Wavelength selection: Di-electrical interference filters. Filter-wheel can hold up to 8 filters; four filters supplied as standard: 405, 450, 492, 620 nm
- Detector: single silicon-photodiode

3.1.2 Measuring process

- Absorbance reader in OD or % Transmission
- Automatic lamp calibration
- Concentration test: comparison value process
- Measured value test: reference value process
- Control measurements: automatic during each measuring operation.

3.1.3 Rated operating conditions

Condition	Specification
Measured wavelength (nm	400 -750 nm
Warming-up time	Ready for operation immediately after switching-on
Operating voltage	90V - 130V, 180V – 250V auto-sensing
Fuses (user exchangeable	2 pcs. 1A TH250VAC, slow blow
Built in Fuse (on power supply):	1 pcs. 2A TH250VAC, fast blow
Ambient temperature	15°C - +40°C (operation) 25°C - +50°C (storage)
Relative humidity	15 - 85% non-condensing (operation) < 95% non-condensing (storage)
Air pressure tolerance	54.000 - 106.000 Pascal
Height over sea level (operation)	up to 2000 m
Installation Category	II
Pollution Degree	2

General technical data

3.1.4 Weight and Dimensions

Weight: 10kg
Dimensions (width x height x length): 34,4 cm x 25,5 cm x 43 cm

3.1.5 Environmental influences

Radio interference according to DIN VDE 0871 in progress: threshold class B for interference voltage, magnetic and electric interference field force
Acoustic power level according to DIN 45 635 part 19: in progress
Outer lighting influences: Precaution, avoid direct sunlight.

3.1.6 Electrical Rating

Consumption: 2010: 50VA, fuse 1A T
2020: 80VA, fuse 1A T
Operating voltage: 90-130V, 180-250V
Frequency range: 47 - 63 Hz (auto-sensing)

3.1.7 Interfacing

Parallel printer interface for IBM or EPSON compatible 9 and 24 pin printer and HP DeskJet 500 series (2020 only).
Serial interface for remote control of basic measurement functions, instrument status and setup by external PC.

General features

- **Absorbency Method:**

  Single channel, well center triggered reading with post-sample filtering, eliminating the influence of ambient light.

- **Measurement Modes:**

  Single- and dual wavelength reading

  Automatic lamp calibration prior to each measurement

  Plate centering system positions any 96-well microplate accurately under the optical path.

- **Quick Measurement Mode:**

  Mono-, bichromatic and kinetic measurement with data output in OD-values on display and printer.

- **Programming and Evaluation:**

  When programming a test the software leads you through a logical sequence of screens, covering all necessary steps.

- **Flexible plate layout functions:**

  Selectable blank validity, auto replicate mode and filling direction. All types of controls and standards can be positioned freely in the plate template.

- **Evaluation possibilities:**

  All evaluation methods can be applied to measurement values (OD), or relational values. Results can be viewed on the screen and outliers rejected prior to printout.

- **Controls and standards:**

  Can either be related to the measured absorbency, the result of a relational calculation, defined by a formula or fixed value. Therefore, fixed or floating cutoffs and standard curves (e.g. single point calibration) can easily be programmed.

- **Powerful formula parser:**

  Measurement values of all controls and standards set in the plate layout as well as constants; variables and mathematical operators can be used to create a formula for calculation of cutoffs, standards

(e.g. preset standard curves, single point calibration) and relational values (e.g. B/B0).

- **Qualitative evaluation:**

  Up to 5 ranges (4 cutoffs) individually named, up to 10 equal sized ranges or %-ranging between 2 cut-off's

- **Quantitative evaluation:**

  Point to point, linear regression, cubic spline, 4-parameter fit, selectable scaling of X- and Y-axis (lin/log)

- **Combined evaluation:**

  All evaluation functions may be combined e.g. quantitative results can be classified using qualitative evaluation.

- **Validation and replicate elimination:**

  Conditions for automatic replicate elimination and control validation may be programmed within a test.

Scope of supply

- Measurement unit
- Serial Cable
- ADAP Basic Software (PC communication program)
- 4 filters: 405, 450, 492, 620 nm
- Dust-cover
- Spare fuses
- Power cord

Installation requirements, Environmental operating conditions

- The working area has to be flat, dry, clean and vibration proof and leave additional room for cables, connections, computer, printer etc.
- The ambient air has to be clean and free of corrosive vapors, smoke and dust. The instrument is rated to Pollution Degree II and Installation Category II.
- The ambient temperature has to range between 15°C and 40°C for operation and between -25°C and +50°C for storage.
- Indoor use only. Humidity has to range between 15% and 85%.
- Height over sea level during operation up to 2000 m

Please Note:
Only use the power cable supplied with the instrument or a power cable with protective earth connection carrying the CE-mark.

The power outlet the instrument is connected to have to meet the applicable technical safety requirements!

If the instrument is turned on using the power switch on the rear side of the device the green power indicator on the left front side of the device has to be illuminated.

When connecting the instrument with an external computer ensure the instrument is switched off (the power indicator is dark). If the instrument is turned on turn it off using the power switch on the rear side of the device.

The power indicator is located on the front left side of the unit and is marked with the following symbol.

The power indicator may show 2 different states:

Green light: Instrument is switched on (ready for operation).

Not illuminated: Instrument is not ready for operation.

Please Note: The power indicator does not indicate that the instrument is turned on or off or that it is connected with mains but only that the instrument is ready for operation!

In order to make sure the instrument is disconnected from power the main switch on the rear side of the unit has to be switched to the "OFF-position" indicated with a "0" symbol. Alternatively the power cord can be disconnected from the unit.

Connecting 2020 with an external computer

Turn the instrument off.

Connect the serial interface (9-pin DB9 connector) on the computer with the serial cable supplied by Biochrom. Lock the connector to the plug using the corresponding screws on the connector.

Connect the other end of the serial cable with the plug marked "RS232" on the rear side of the instrument. Lock the connector to the plug using the corresponding screws on the connector.

Please Note: Only use the original cables supplied by Biochrom.

Alternatively, the instrument can be connected to a computer using a RS232 to USB adaptor. Please contact support@biochrom.co.uk for recommended RS232 to USB adaptors.

Re-packing to prevent damage during transport

The original packing has been specially designed for this equipment. It is recommended to save the original carton with its foam parts and accessories box. Warranty claims are void if improper packing causes transport damages!

4 Warranty

A warranty period of 12 months shall be granted to the original buyer of the Biochrom Anthos 2020. This warranty shall lose effect if:

- Biochrom Anthos 2020 is not used in the defined scope of application
- Biochrom Anthos 2020 has obviously been damaged by external influences which are not in accordance with the provisions for the nominal range of use,
- Biochrom Anthos 2020 has been modified or parts exchanged by a person other than Biochrom personnel or an authorized servicing agent,
- The warranty seals on the housing of the instrument are broken,
- parts and subassemblies are implemented, which are not original from Biochrom,
- Biochrom Anthos 2020 serial number is no longer legible, has been removed or altered,
- Biochrom Anthos 2020 has not been installed in accordance with the instructions supplied,
- Biochrom Anthos 2020 has been damaged during return transport due to wrong packing (e.g. not in original packing material).
- Biochrom Anthos 2020 was damaged due to wrong operation, not according to the descriptions in the manual.

If an instrument is under warranty, Biochrom shall repair or replace any defects, which have resulted from faulty material or during production as it sees fit. No costs shall arise for the client (except cargo rates).

All components found in the original equipment, or an adequate and full compatible alternative shall be available for a period of 5 (five) years after production.

This warranty refers to the obligations of Biochrom and can only be amended upon the written consent of Biochrom.

Liability

In its original condition the instrument meets all safety regulations for a risk-less operation.

Biochrom cannot be liable for damages or any resulting costs caused by unauthorized alterations, repairs or modifications of the equipment.

5 Installation

Un-packing and System Set Up

The original packing has been especially designed to protect the instrument during transportation. It is therefore recommended to keep the original carton with its foam parts and the accessories box for re-use in case of future shipments. Warranty claims are void if improper packing causes transport damages!

Check the box for any visible damage during transportation. In case of damage inform your supplier immediately and keep the damaged packing

- Place the device on a suitable working surface
- Remove the transportation lock (foam part) from the plate holder
- Connect the serial cable to PC
- Connect power cable to standard mains plug

Power On

The Biochrom Anthos 2020 performs the complete initialization and shows the main menu after approx. 30 seconds.

Software Installation

5.1.1 ADI and ADAP

ADAP Basic is remote control software for Anthos instruments and is supplied as standard with Biochrom Anthos 2020. ADAP Plus and ADAP Expert are evaluation software packages offered by Biochrom, Ltd.

- Insert the CD-ROM and open Start- Here.html on the CD
- Install ADI and ADAP software as described in this file
- Connect instrument and PC with the serial cable provided with the instrument.
- Start ADAP and set up the instrument in ADAP Software
- All further steps are described in the ADAP User Manual

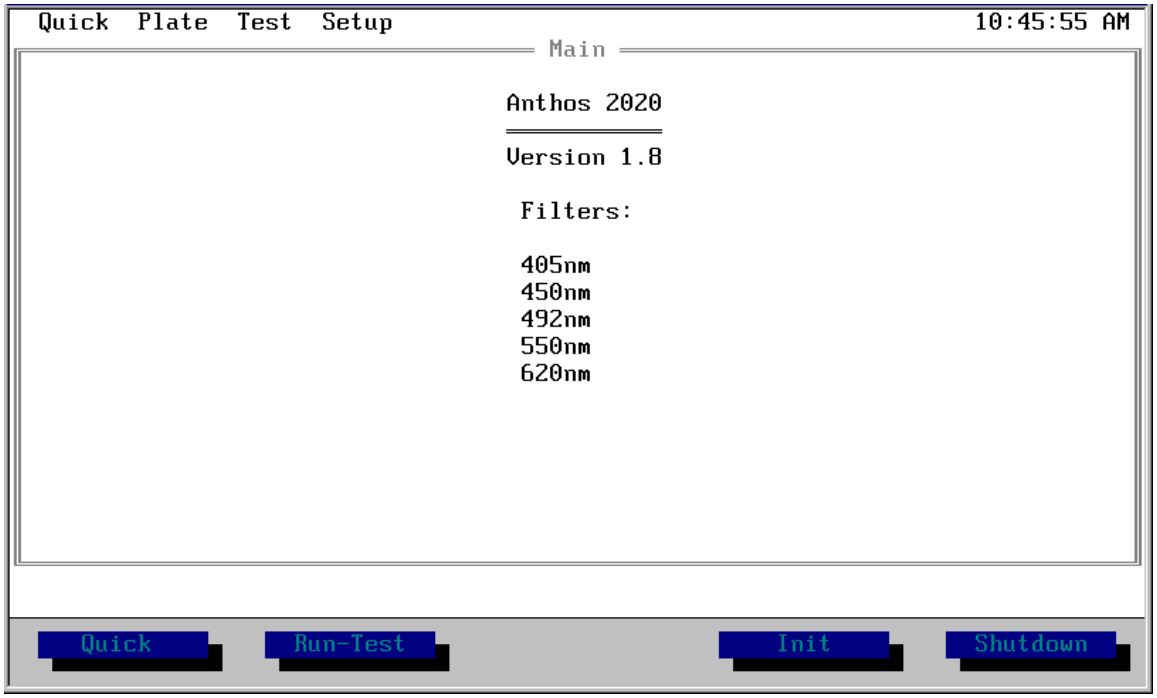
Switching off

When switching off the Biochrom Anthos 2020, make sure that the command F5 - Shutdown (or “Shutdown” in the “Setup” menu) is always used. If this is not used, specific settings will NOT be stored. Wait for the message “Switch the instrument off”, and then turn off the unit with the mains switch!

6 2020 On-Board Software

Controls and System Setup

6.1.1 Description of the key functions



<stop> - during a measurement or in the test definition this key aborts the current task. It can also be used to cancel dialogues

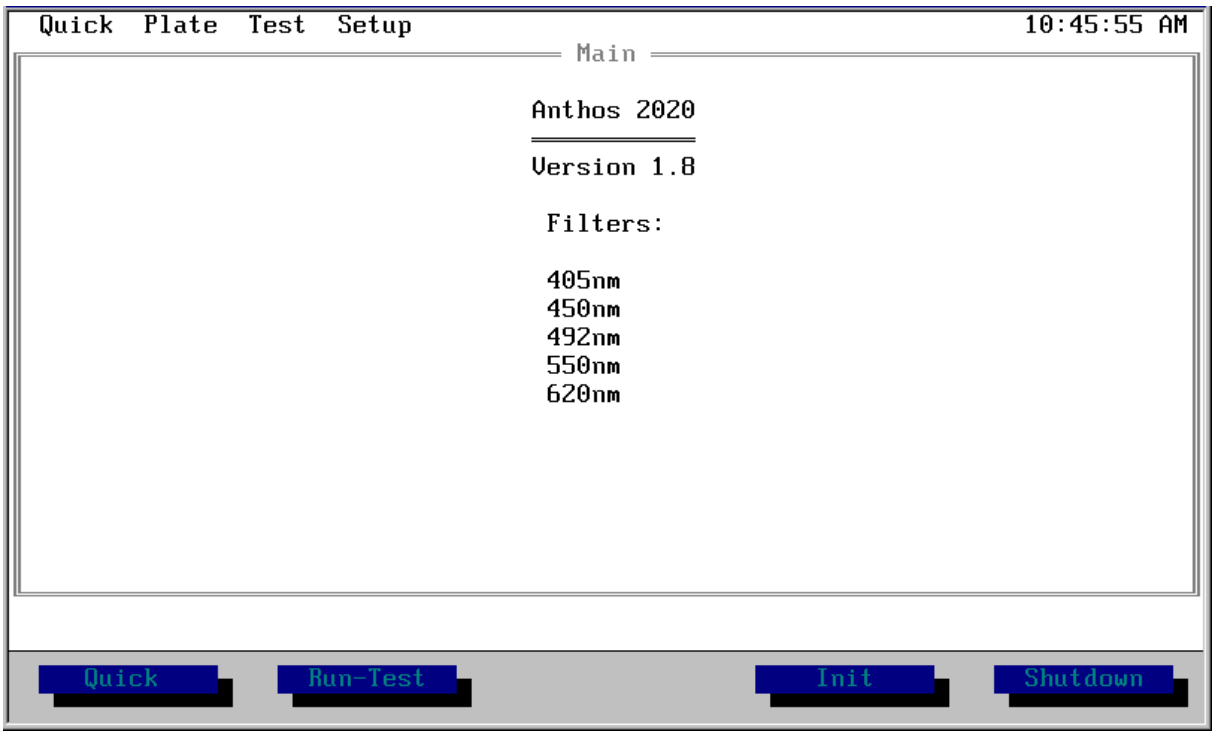
<prev> - back to the previous dialogue element

<enter> - confirms the text / numerical entry and leads to the next dialogue element

<ce> - for changing and editing existing values and for activation of the text and/or formula input.

<display> - holding this key and pressing the cursor up or down keys in- or decreases the display contrast.

6.1.2 **Display**



Screen 2.: Main Menu

Options:

Press <menu> and use the cursor keys to select menu options

Function keys:

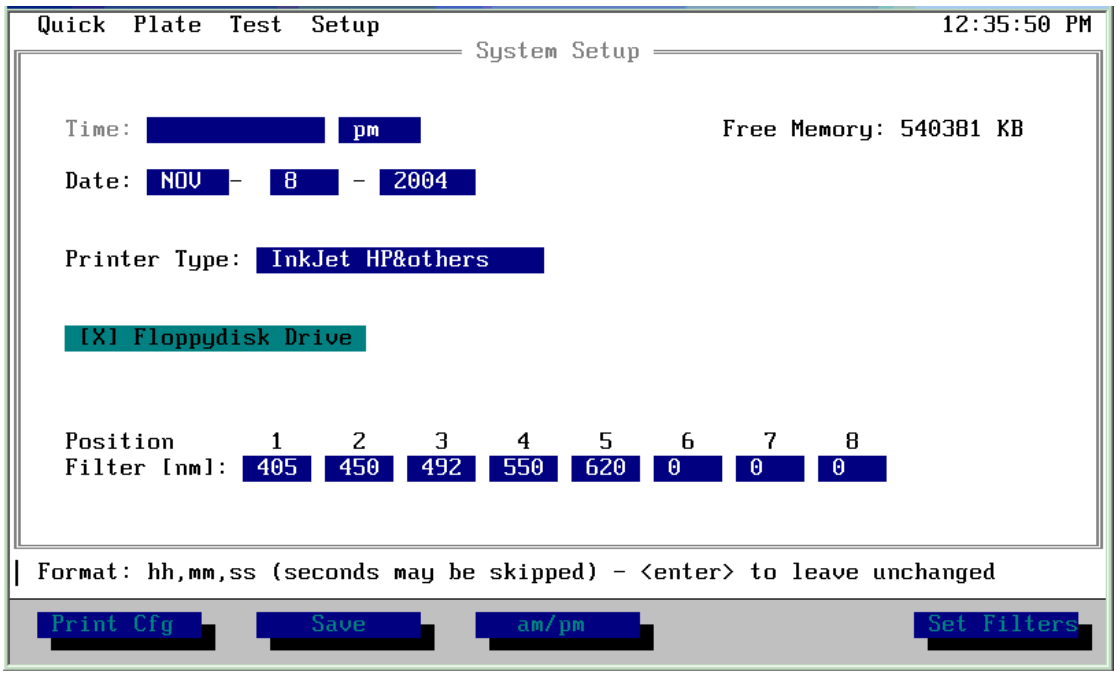
F1 – QUICK: Start a quick measurement without test programming

F2 – RUN-TEST: Start a pre-programmed test

F4 – INIT: Perform an initialization of the instrument

F5 – SHUTDOWN: Prepares the instrument to be turned off

6.1.3 System Setup Functions



Screen 3.: System Setup

In the main menu press <menu> and select “Setup” and “System”.

Options:

Date and time (Useful for print-outs)
Free Memory: shows the amount of free (available) space on the hard drive or flash disk

Printer Types:

- Matrix 9-pin
- Matrix 24-pin
- Inkjet Canon & Epson
- Inkjet HP & others
- Laser
- IBM Proprinter
- Postscript
- HP Paint jet
- HP Think jet

Floppy Disk Drive: to activate/deactivate press <ce>, an activated Floppy Disk Drive is marked with [X]. To activate functions regarding to “data management using disks, restart the machine with activated Floppy Disk Drive.

Filter position and wavelength: To change, delete wrong entry with CE and enter new value using the numerical keyboard.

Function keys:

F1 – Print Cfg-Print the configuration of the instrument

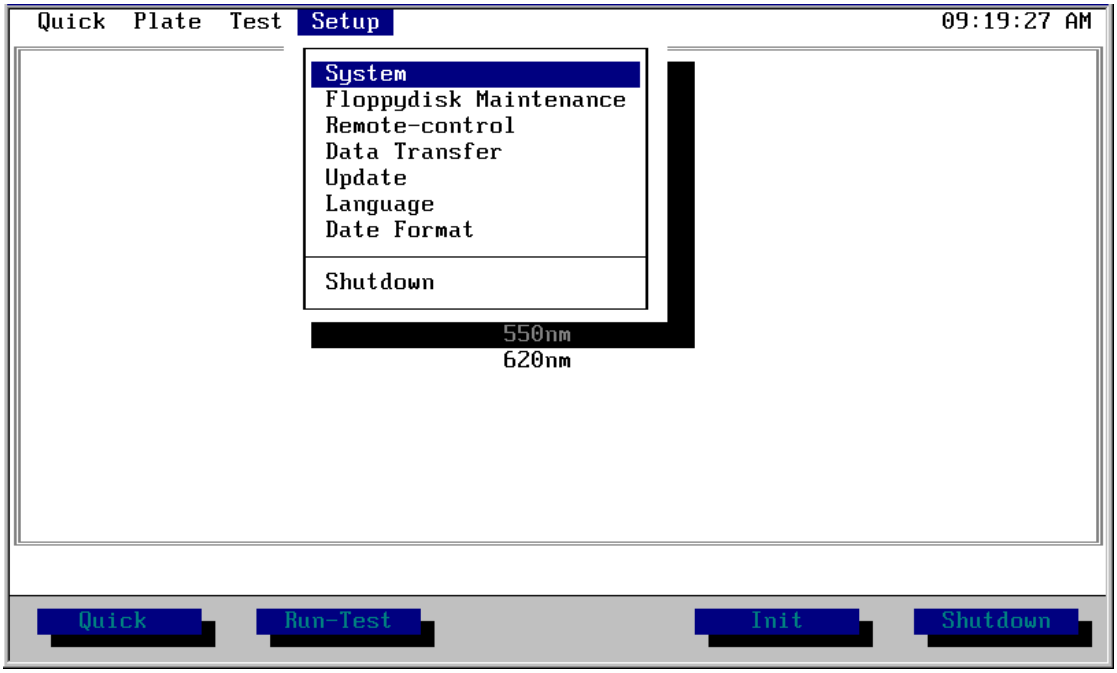
F2 – Save

F3 – am/pm-Switch between am and pm
(only when Date Format is set to 12 hour mode, see below)

F5 – Set Filters-Perform each time the filter wheel has been changed to ensure that the correct filters are installed and that the lamp energy is correct.

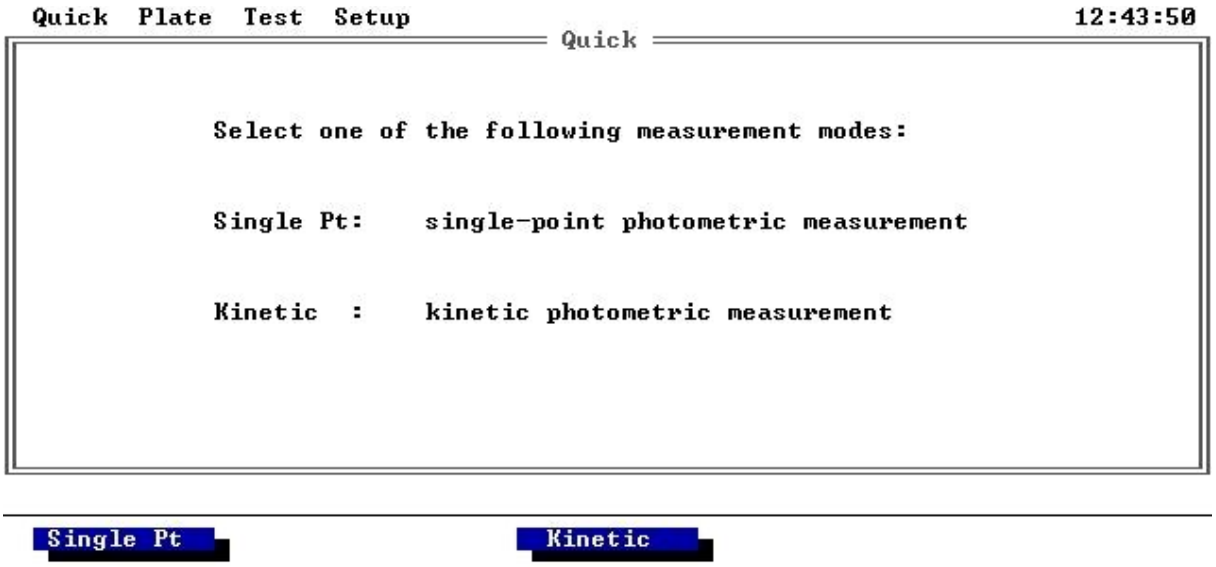
6.1.4 Setup Menu Options

- Floppy-disk Maintenance: Refer to chapter 6.1.36
- Remote control: Instrument can be controlled via PC.
- Data Transfer: Data-transfer via ADAP (Data-storage, up- and download of tests and plates on instrument or PC).
- Update: For software-update of the 2020.
- Language: You can choose between German and English language.
- Date format: Select between 24 h and 12 hour am/pm.
- Shutdown: Press this button for shutdown.



Screen 4. Additional options of the setup menu

QUICK measurements without test programming and storage



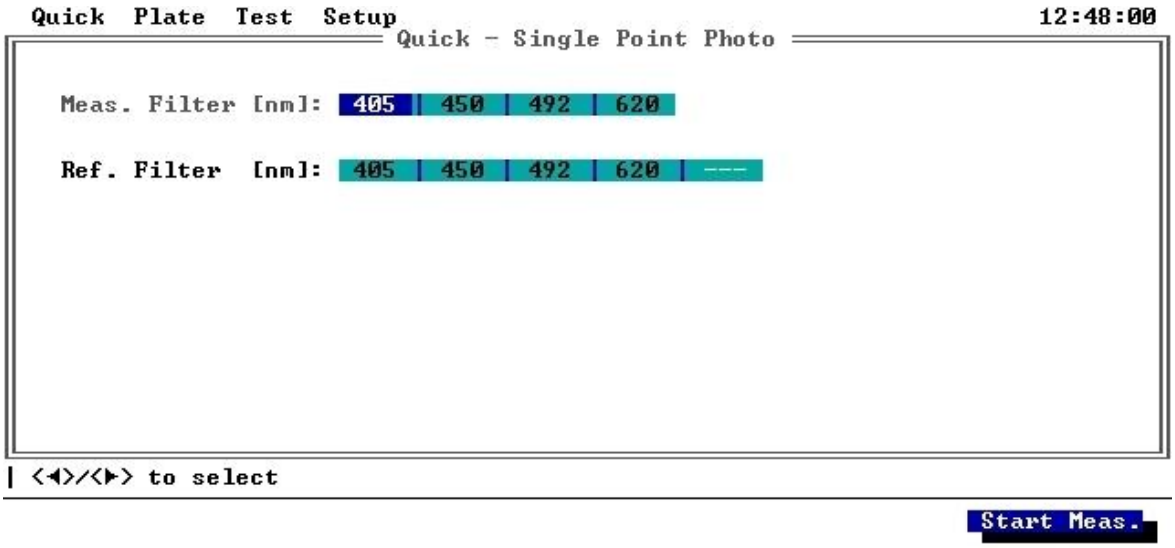
Screen 4. Quick Menu

Function keys:

F1 – Single Pt: Perform a single point, mono- or bichromatic absorbency reading

F3 – Kinetic: Perform a kinetic absorbency reading

6.1.5 F1 – Single Point



Screen 5. Single Point Measurement

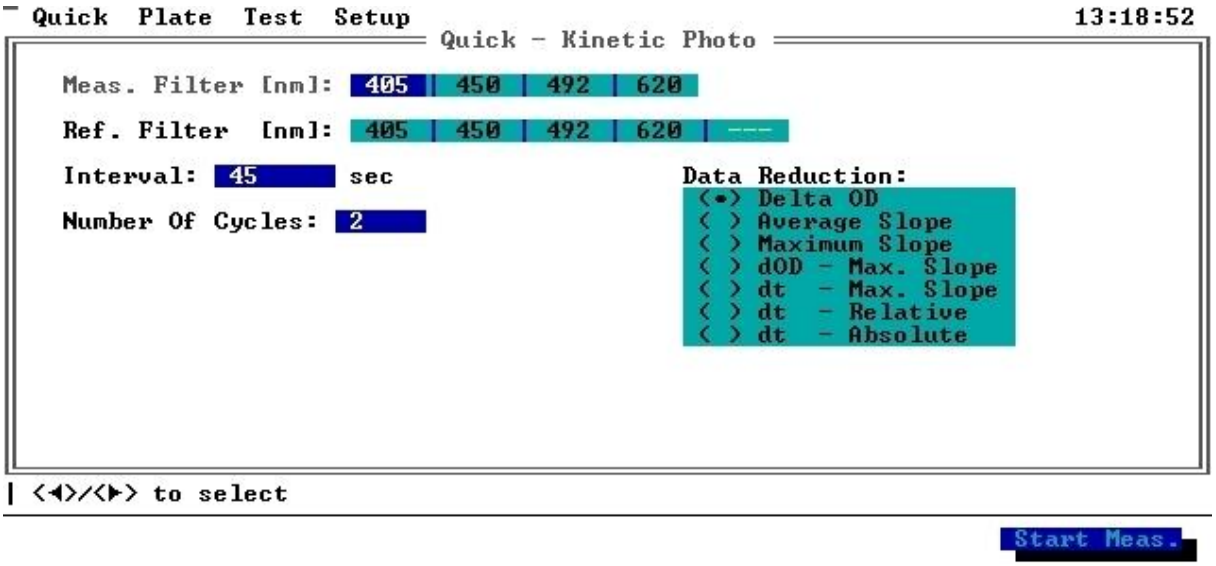
Options:
Measurement filter: highlight the desired measurement filter and confirm with **<enter>**

Reference filter: highlight the desired reference filter (as above; if no reference filter is required, select "---")

Function keys:

F5 – Start Meas: Start the measurement with the selected filter(s).

6.1.6 F3 – Kinetic



Screen 6. Kinetic Measurement

Options:

Measurement filter: highlight the desired measurement filter and confirm with <enter>

Reference filter: highlight the desired reference filter (as above; if no reference filter is required, select "---"); Usually no reference filter is used for kinetic data since a reference reduction only shifts the relevant reaction process up or down, which has no effect on the results of the data reduction.

Interval: interval setting in seconds from the beginning of one measurement cycle to the beginning of the next cycle (minimum time = 45 seconds for single wavelength, 90 seconds for dual wavelength).

Number of cycles: number of measurement cycles to be performed (press <ce> and enter number using keyboard).

Data Reduction:

Delta OD

Average Slope

Maximum Slope

dOD – Max. Slope

dt – Max. Slope

dt – Relative

dt – Absolute

Smoothing Points:

Depending on the selected data reduction mode, smoothing points can be defined: To reduce peaks in the result, a number of values can be defined, of which an average or slope (depending on the data reduction mode) is calculated (e.g. if 3 smoothing points are selected, an average or slope for the values 1, 2 and 3 is calculated, then the average or slope for the values 2, 3 and 4 is calculated, etc.).

Function keys:

F5 - Start Meas: By pressing this function key the measurement will be started with above defined parameters.

6.1.6.1 Data Reduction Methods

Data Reduction is used to determine a single value per sample based on the results of a sequence of measurements over a period of time.

Delta O D: Difference between two kinetic measurements in [O D]

Average Slope: Slope in [O D/min] between the first and last kinetic cycle. Only suited for linear reaction progressions with few cycles and measurement points.

Maximum Slope: Maximum slope of the curve in [O D/min]. (Maximum reaction speed). The line with the highest slope is calculated. The accuracy of this calculation depends on the number of measurement cycles selected.

Delta O D - Maximum Slope: Difference in [O D] between the first measurement and the center point of the maximum slope. Calculation of the center point of the maximum slope: Center point between the four measurement points of the regression line with the maximum slope.

Delta t - Maximum Slope: Time difference in [sec] between the first measurement and the occurrence of the center point of the maximum slope.

Delta t - Relative: Time in [sec] from the first measurement to reaching a set increase/decrease in m [O D].

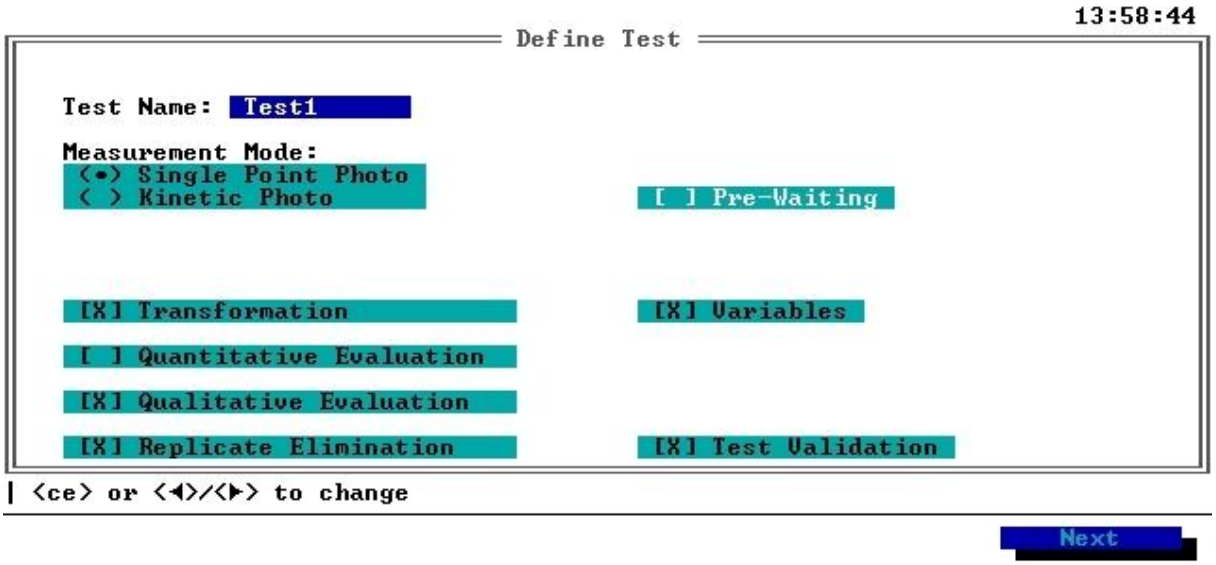
Delta t - Absolute: Elapsed time from one pre-selected threshold value to another.

Function keys:

F5 – Start Meas.: Start the measurement with the selected filter(s).

Test Definition - Programming of tests (evaluation modes)

Press <**menu**> and select "**Test**" and then "**New**" to define a new test.



Screen 7.: Define Test

Since all programming options together are seldom used during one test, optional settings may be skipped. If a special point, e.g. "quantitative evaluation" is required, the question regarding the use of such function must first be answered checked [X] (press CE) before the corresponding menu (e.g. "standard points" and "quantitative evaluation") is displayed.

6.1.7 Test Identification and Pre-Measurement Functions:

- Test Name: Press <ce> to activate the text input mode. Now the text position (left, right) and the required letters (up, down) can be selected with the cursor keys.

- Measurement Mode: Select the measurement mode:

  ✓ Single Point Photo
  ✓ Kinetic Photo

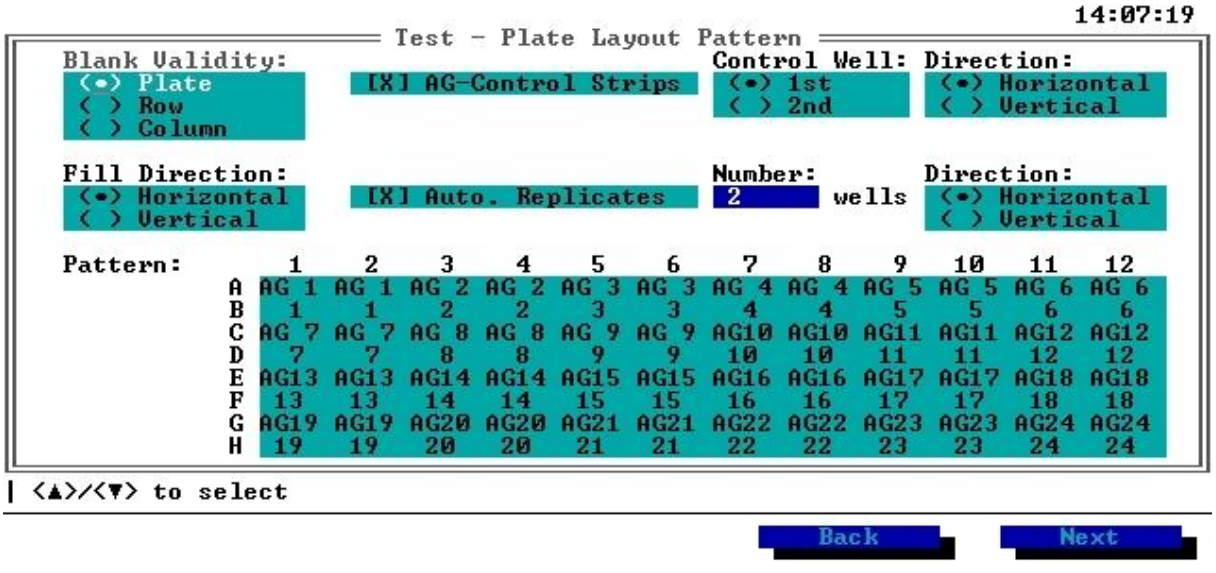
- Transformation: Mark [X] this option if relational calculation is required (all measurement values are re-calculated with a defined formula; please refer to chapter 6.1.14 "Relative Calculation")

- **Quantitative Evaluation**: Mark [X] this option for quantitative evaluation.
- **Qualitative Evaluation**: Mark [X] this option for qualitative evaluation.
- **Replicate Elimination**: Mark [X] this option if automatic replicate elimination is required.
- **Pre-Waiting**: Mark [X] this option to activate a waiting time before the measurement starts (e.g. for kinetic measurements where the reaction starts only after a certain time).
- **Variables:** Mark [X] this option if special variables for calculations are to be defined (e.g. for the definition of lot specific factors or concentrations).
- **Test Validation**: Mark [X] this option if an automatic test validation is wanted. The criteria for the validation can be defined later on.

Function keys:

F5 - Next
Proceed to the next screen.

6.1.8 Plate Layout Pattern



Screen 8.: Plate Layout Pattern

Options:

Blank Validity: Validity of the blank value positions on the plate:

Plate: Mean value of all individual blanks is automatically deducted from all other measurement results-RECOMMENDED

Row: Free configuration of the blanks on the entire plate. The mean value of all blanks in each row is deducted from each sample of this row.

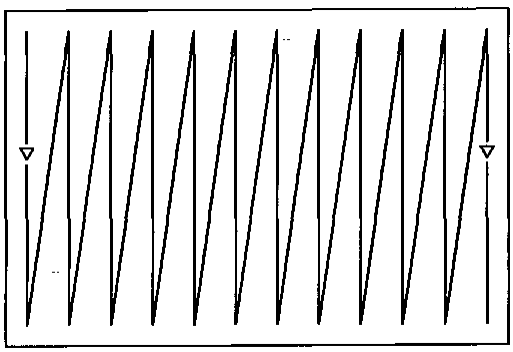
Example: *Setting blanks on plate position A01 and A08.*
Mean value of A01 and A08 = (A01+A08)/2
this mean value is deducted from each sample of this row (A02/A03/A04/A05/A06/A07/A09/A10/A11/A12).

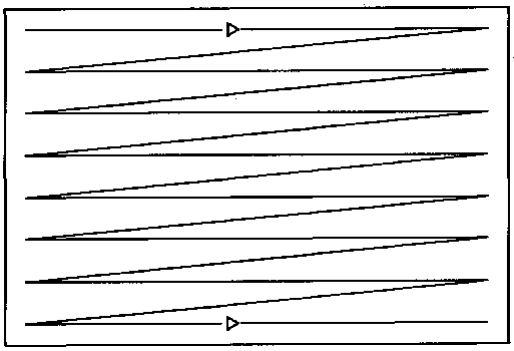
Column: Free configuration of the blanks on the entire plate. The mean value of all blanks in each column is deducted from each sample of this column.

Example: *Setting blanks on A01, B01 and D01. Mean value of A01, B01 and D01 = (A01+B01+D01)/3*
this mean value is deducted from each sample of this column (C01/E01/F01/G01/H01).

Fill Direction: Filling/pipetting direction (horizontal/vertical).

Note*: Groups of replicates are sorted according to their replicated direction AND the filling direction.*





AG-control strips: Antigen control strip setting. A control sample, which is automatically deducted, is assigned to each sample. The AG-control-strip is always labeled AGC on the

printout. The control well sample positioning depends on the filling direction and the sample control setting combination of the reader.

Control Well: Select whether the control well is located before or after the sample

Direction: Direction (horizontal/vertical) of the AG-control-strips relative to the sample (if AG-control-strips and replicates are used together, the filling direction for the replicates is automatically set according to the filling direction of the AG-control-strips).

Auto Replicates: automatically set replicates, valid for all samples, blanks, controls and standards on the plate. Set the following parameters:

Number: Number of replicates (maximum of 12 replicates for horizontal direction; maximum of 8 replicates for vertical direction)

Direction: Direction (horizontal/vertical) of replicates (if AG-control-strips and replicates are used together, the filling direction for the replicates is automatically set according to the filling direction of the AG-control-strips).

Function keys:

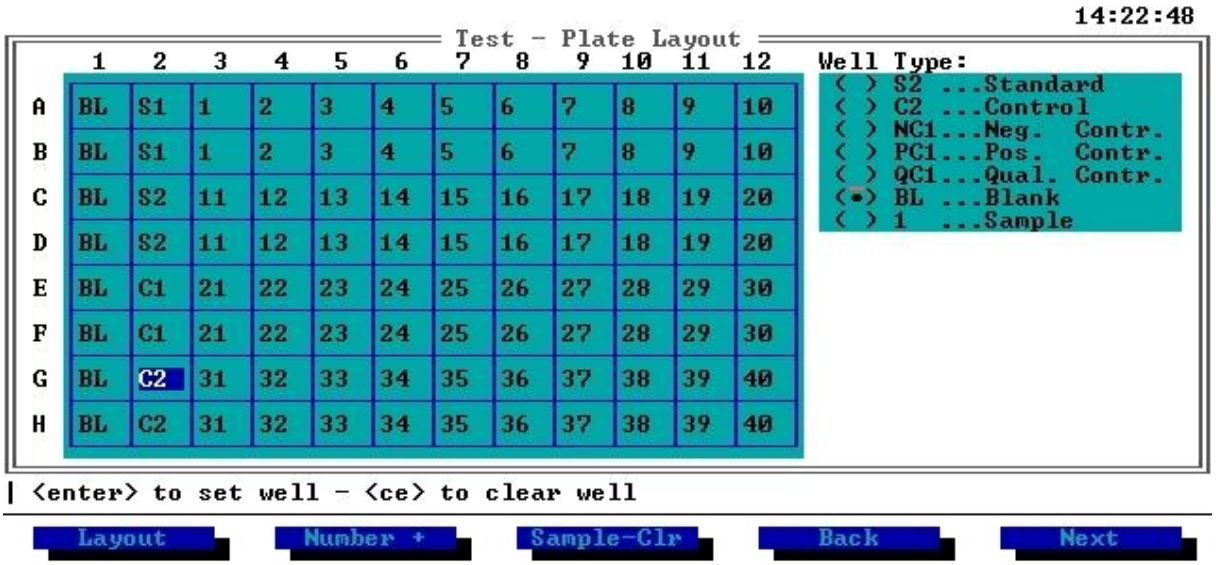
F4 – Back

Return to the previous screen.

F5 - Next

Proceed to the next screen.

6.1.9 **Plate Layout**



Screen 9.: Plate Layout

Options:

Well Type: Select one of the possible well types (Standard, Control, Blank,...). Press **<F1 – Layout>** to jump to the plate layout, position the cursor into the desired well and press <enter> to define this well as the selected well type. To select a different well type press <F1 – Well Type> and select with the cursor keys.

In the well type window the ID-number of the standard, control, etc... can be increased by pressing <F2 – Number +>.

Function keys:

F1 – Layout/Well Type

By pressing this key the cursor can be set from the Well Type window into the Layout window and vice versa. To place the selected well type in the highlighted position of the layout, press the enter key.

F2 – Number +

Press this key to increase the ID-number of the selected well type (control, standard, sample,...).

F3 – Sample-Fill (Sample-Clr)

Press this key for automatic sample positioning. The vacant positions are filled with samples in the prescribed filling direction. After filling all positions, the function key will change to Sample-Clr, which allows to clear all samples set previously.

F4 – Back

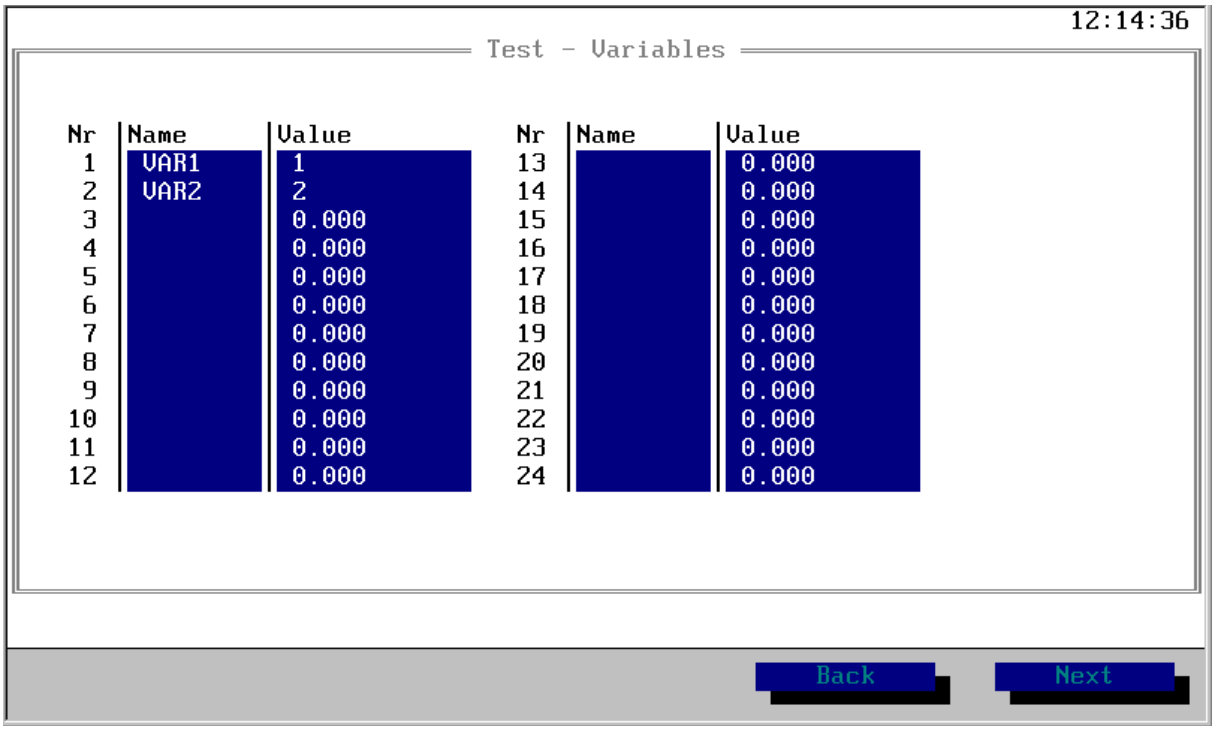
Return to the previous screen.

F5 - Next

Proceed to the next screen.

6.1.10 **Variables**

For the evaluation of measurement results it is sometimes necessary to use lot dependent factors or concentrations (e.g. lot dependent titers of standards). For this reason up to 24 variables per test can be defined and may be used for further calculations.



Screen 10.: Variables

Options:

Name: Define a name for the desired variable by pressing <ce> and enter the name with the cursor keys.

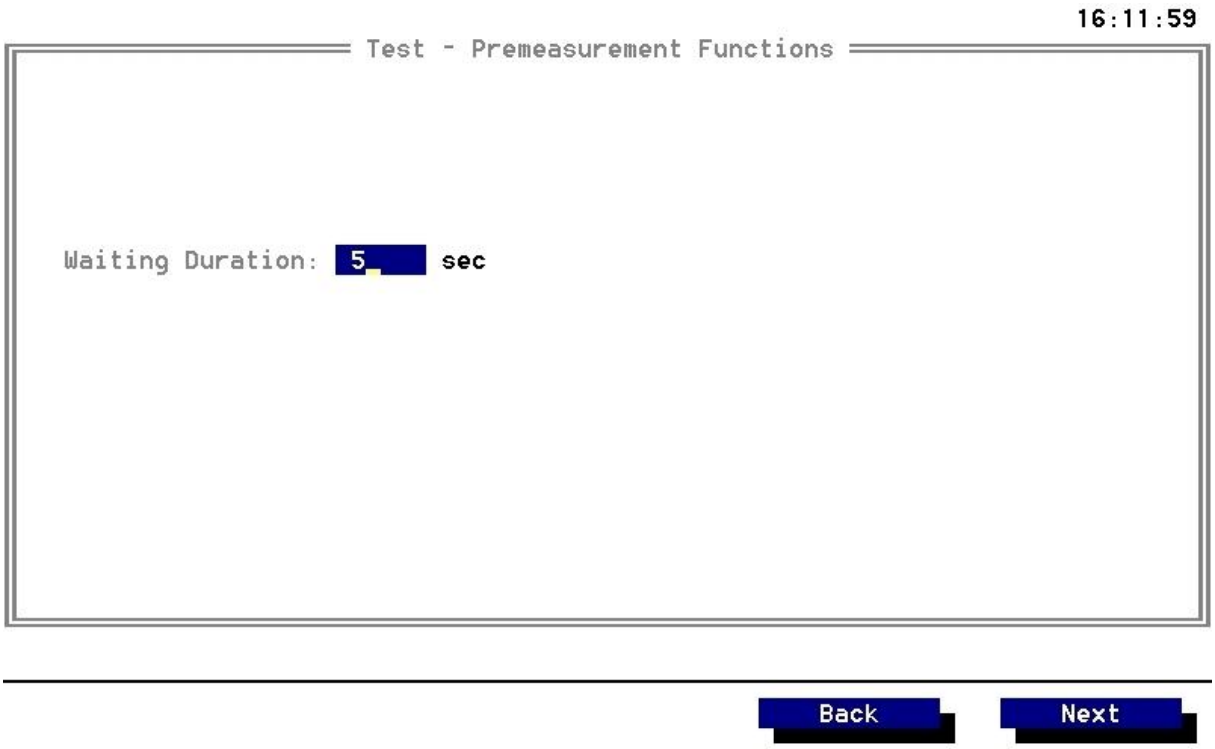
Value: Enter the value for the selected variable.

Function keys:

F4 – Back
Return to the previous screen.

F5 - Next
Proceed to the next screen.

6.1.11 **Pre-Measurement Functions**



Screen 11.: Pre-measurement Functions

Options:

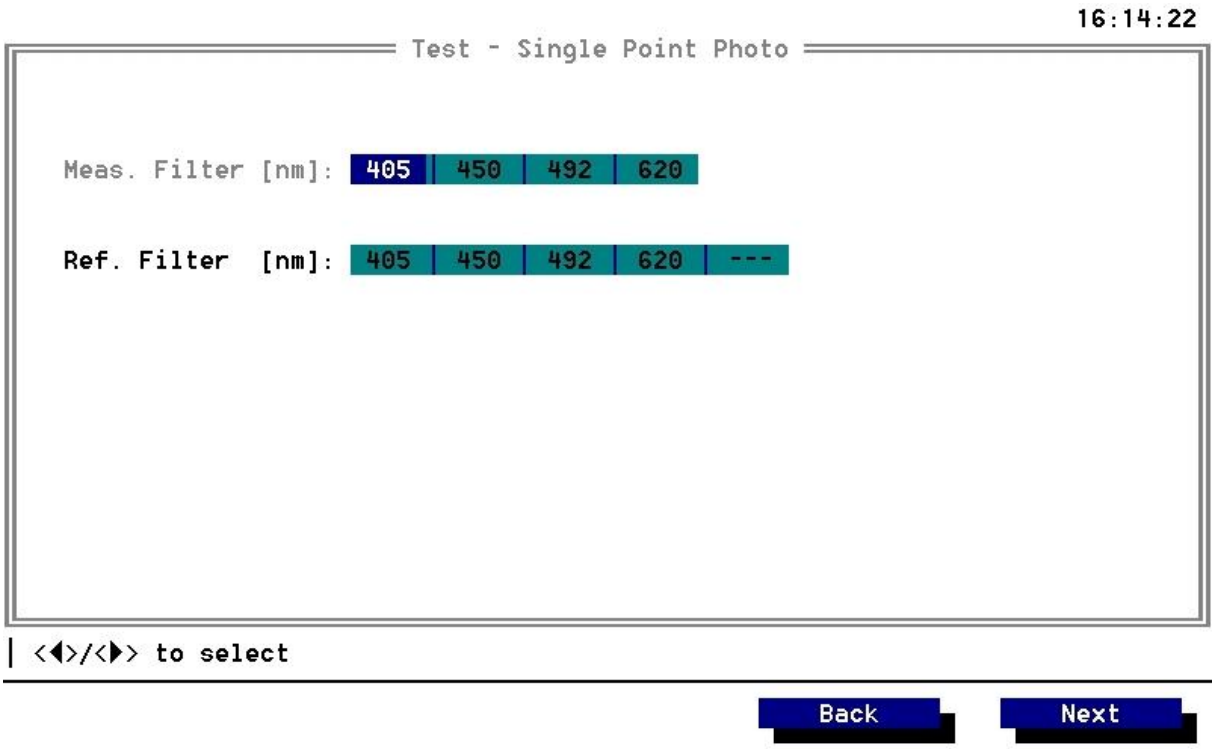
Waiting Duration:
Enter the waiting time (waiting prior to measurement) in seconds (1 – 999).

Function keys:

F4 – Back
Return to the previous screen.

F5 – Next
Proceed to the next screen.

6.1.12 **Wavelength Selection**



Screen 12.: Single-Point-Photo

Options:

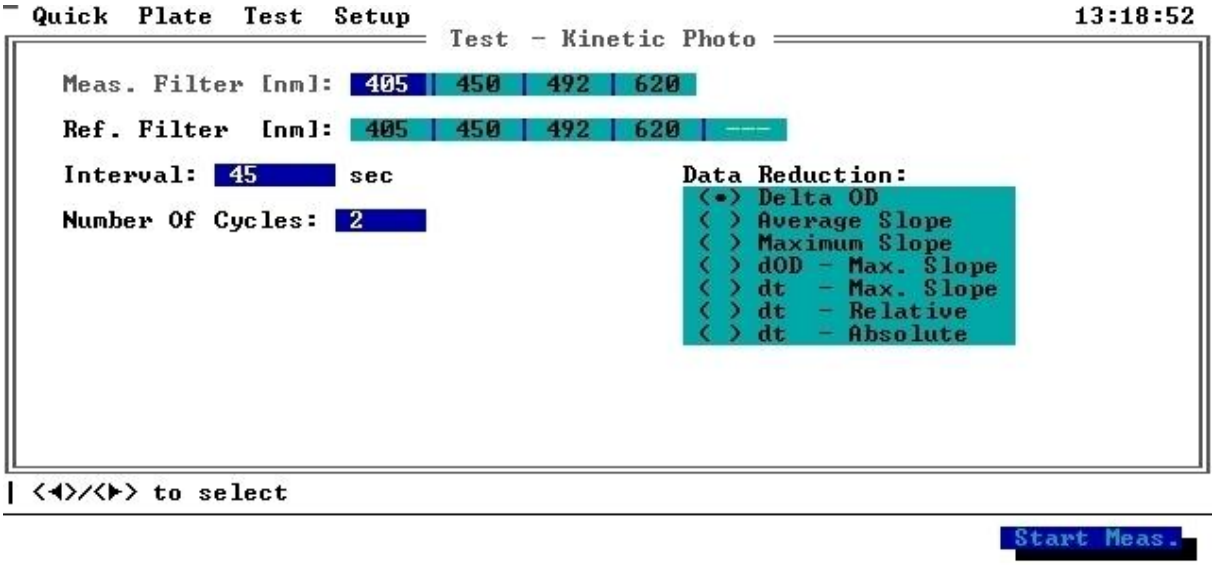
Please refer to chapter 6.1.5 F1 – Single Point for detailed information on all settings.

Function keys:

F4 – Back
Return to the previous screen.

F5 – Next
Proceed to the next screen.

6.1.13 **Kinetic Absorbency Measurement**



Screen 13.: Kinetic Photo

Options:

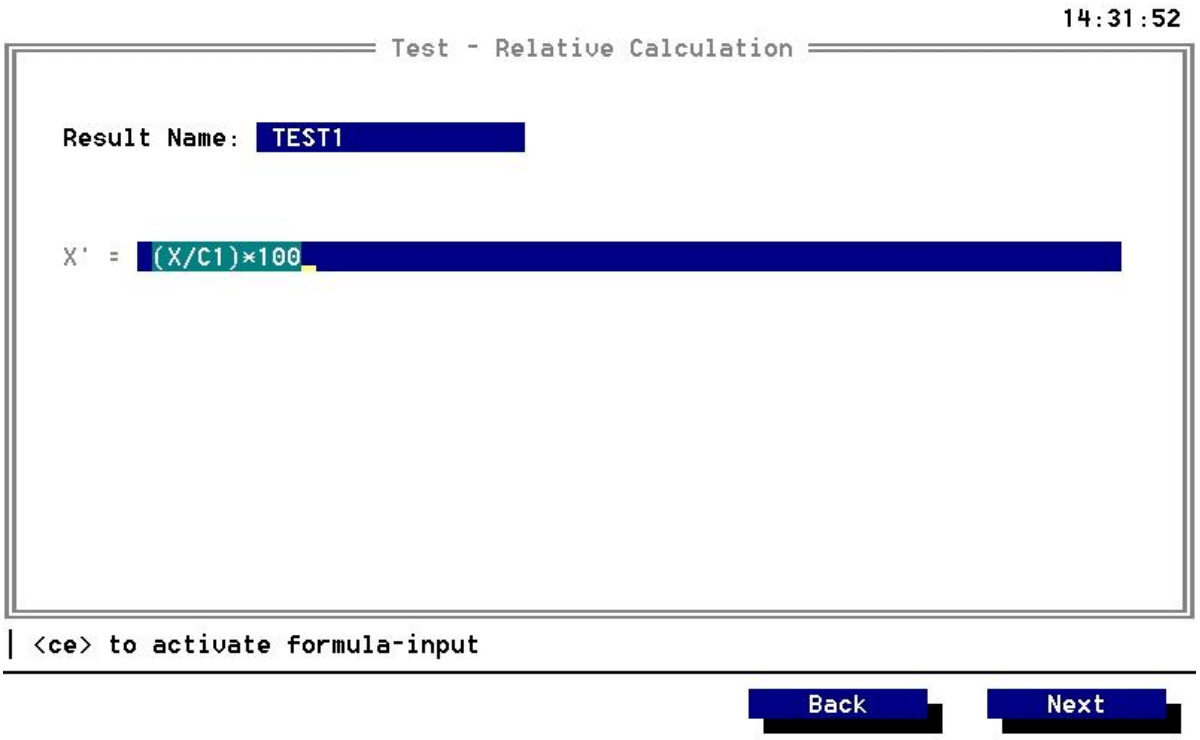
Please refer to chapter 6.1.6 for detailed information on all settings.

Function keys:

F4 – Back
Return to the previous screen.

F5 – Next
Proceed to the next screen.

6.1.14 **Relative Calculation**



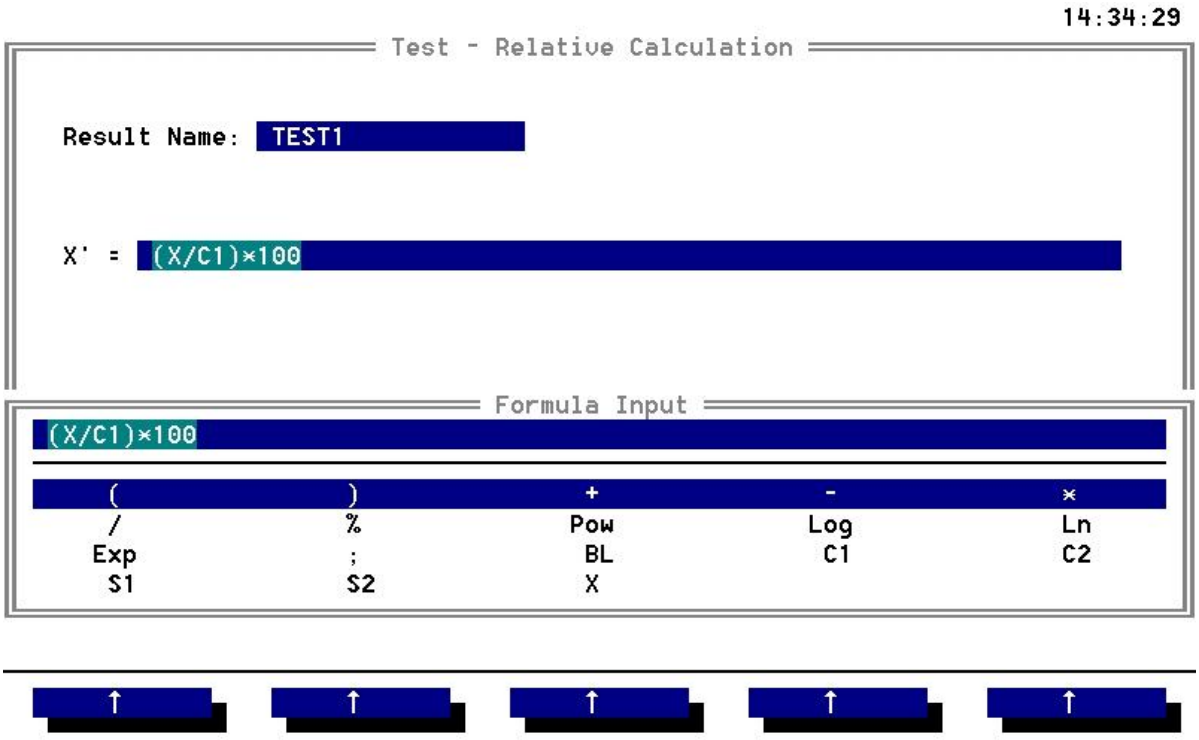
Screen 14.: Relative Calculation

Options:

Result Name
Activate the text input with **<ce>** and enter a result name.

Formula
Sets all plate positions in relation to a predefined value.

This value is calculated according to an algebraic formula, which may contain all the standards, controls and blanks defined in the plate layout as well as the fundamental operations of arithmetic and numerical constants and the previously defined variables. Press **<ce>** to activate the formula input.



Screen 15.: Formula Input

The display menu shows all the standards, controls, blanks and variables defined in the plate layout and the pre-measurement functions as well as a variable X. This variable X represents all the original measurement values. It must appear exactly once in each algebraic formula. Move the cursor to select a row in the screen display and select the desired character with the corresponding function key. Numbers may be entered on the numerical keyboard.

Example:

100% Binding is equal to the absorbance of NC1, binding of samples is expressed as a ratio of NC1. The equation should be written as:

= (X/NC1)*100, X is the measurement value of the well

Additional mathematical functions

The software includes the following 4 additional mathematical functions to be used in formula definition:

Mathematical Functions	
Pow(x;y)	$_xy$
Log(x)	$\log_{10} x$
Exp(x)	e^x
Ln(x)	ln x

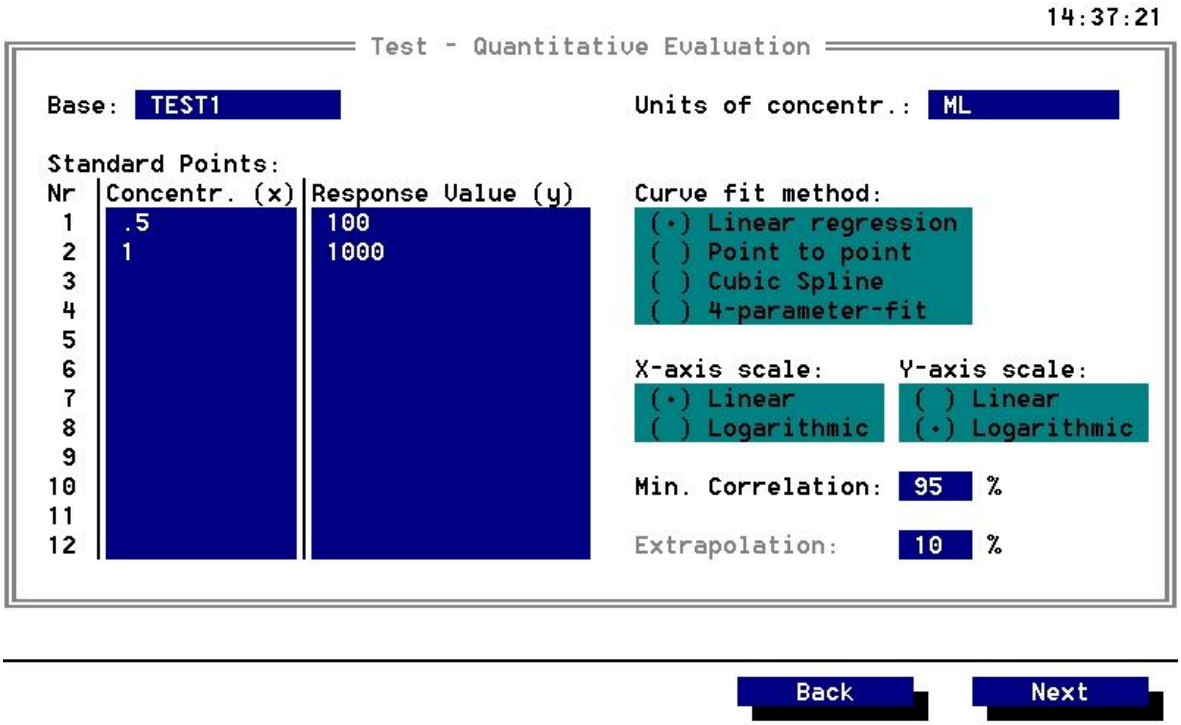
Function keys:

F4 – Back
Return to the previous screen.

F5 – Next
Proceed to the next screen.

6.1.15 **Quantitative Evaluation**

This method obtains sample concentrations using a standard curve.



Screen 16.: Quantitative Evaluation

6.1.15.1 **Set-up a Standard Curve.**

Options:

Base:
Select the calculation base (y-values) for the concentration calculation: If a Relative Calculation formula is set up, the original measurement values (default) or transformed values (results of the relative calculation) may be selected. The name for transformed values refers to the 'Result Name' defined in chapter 6.1.14 .

Concentration (x):
Enter the concentration value to be plotted on the x-axis of the curve graphic. Constants of up to 12 digits may be entered on the numerical keyboard. It is also possible to define formulas containing all the set controls, standards, numerical constants and variables for the concentration value.

To enter a new formula press <ce> scroll, with the cursor keys up and down and select the required operator / variable with the corresponding function key.

The concentrations of the standards have to be entered in increasing order!

Response value (y):
Refers to the absorption value to be plotted on the y-axis of the curve graphic. Its default formula is the identifier of the standard points (S1, S2, ...) and corresponds with the OD-values of the standards on the plate. However it can also be defined by an algebraic formula containing all the set controls, standards, numerical constants and variables. To enter a formula press <ce>, scroll with the cursor keys up and down and select the required operator / variable with the corresponding function key.

Units of Concentration:
Press <CE> and enter a name by scrolling through the alphabet.

Curve Fit:
Select a suited curve fitting formula. Depending on the type of curve calculation, a minimum number of standards must be set:

Linear regression	2 standards
Point to point	2 standards
Cubic spline	3 standards
4-parameter-fit	4 standards

Maximum number of standards: 12.

See chapter 6.1.15.2 below for detailed description of curve fits.

Scaling:
Select linear or logarithmic scaling for x-axis and y-axis.
If the sequence of standard concentrations is produced by a multiplication factor (e.g. 2, 4, 8, 16, ...), set the x-axis logarithmic. If the sequence is produced by a constant increase (e.g. 2, 4, 6, 8, 10), then the x-axis is linear. The y-axis will be linear except in cases of extreme dynamic ranges. The best scaling setup is the one, which produces the most linear curve at medium (close to 45°) slope.

Please note that it is not possible to use a log-scale on x-axis, if the first standard has concentration zero (for mathematical reasons).

Minimum Correlation:
This refers to linear regression only and is a limit for the standards to fit a straight line. A good linear curve should be better than 98.0 %. The best fit is 99.9 %. Enter a value between 1 and 99.9 on the numerical keyboard and confirm with <enter>.

Extrapolation:
The concentration of samples having a higher / lower measurement result than the highest / lowest standard can be determined mathematically. A continued calculation of the curve can be performed up to 40% outside the real curve limits.

This percentage corresponds to the concentration range between the lowest and highest standard (=100%). Extrapolated values are marked with an "X" on the printout. If a curve loses its uniformity in the extrapolation range (it reaches its maximum or minimum), extrapolation is blocked.

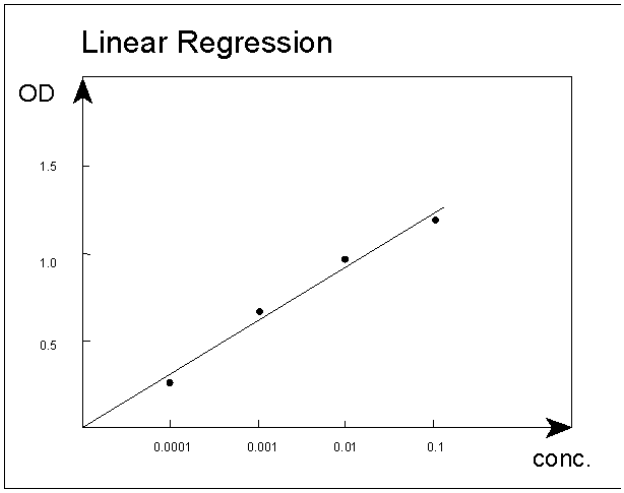
Enter a value between 0 and 40 on the numerical keyboard and confirm with <enter>.

6.1.15.2 Curve Fit Functions:

Four different mathematical functions are available to calculate the standard curve. It is essential to select the best fitting function for the standard points, according to the test:

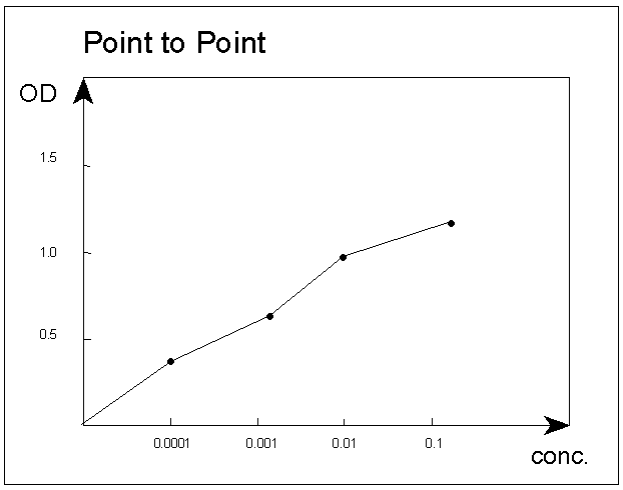
1. Linear regression:
   A straight line is fit through the data using the least squares method with the highest possible approximation to all standard points.

   Minimum number of defined standard points: 2
   Formula: y=kx+d



Drawing 17.: Linear Regression

2. Point to point:
   Direct connection of all standard points.
   Minimum number of defined standard points: 2



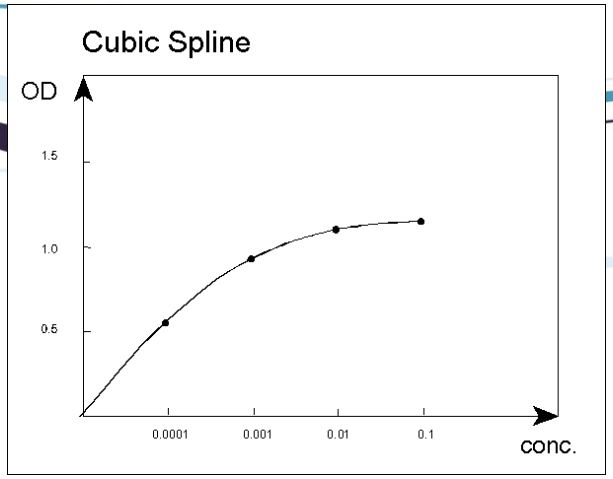
Drawing 18.: Point-to-Point

3. Cubic spline:
   All standard points are connected by the best fitting curve. Can only be used for non-linear and non-sigmoid functions.

   Minimum number of defined standard points: 3

   Definition of the “natural cubic spline” as found in: G. Engeln-Müllges, F. Reutter: “Formelsammlung für numerische Mathematik mit Pascal-Programmen”, Bibliographical Institute, Mannheim, Vienna, Zurich; Wissenschaftsverlag, 1985; p146ff.

$$y_i = \frac{(a-d)}{[1+(\frac{x_i}{c})^b]} + d$$



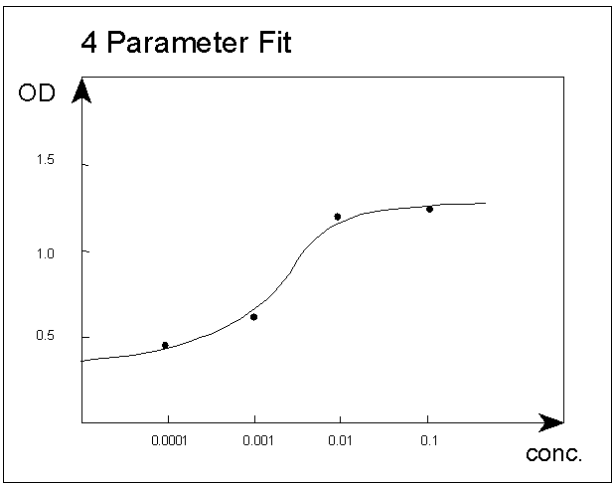
Drawing 19.: Cubic Spline

4. 4 Parameter Fit:
   This procedure can only be used to characterize sigmoid curves.
   Minimum number of defined standard points: 4

   The curve is calculated according to the following formula:

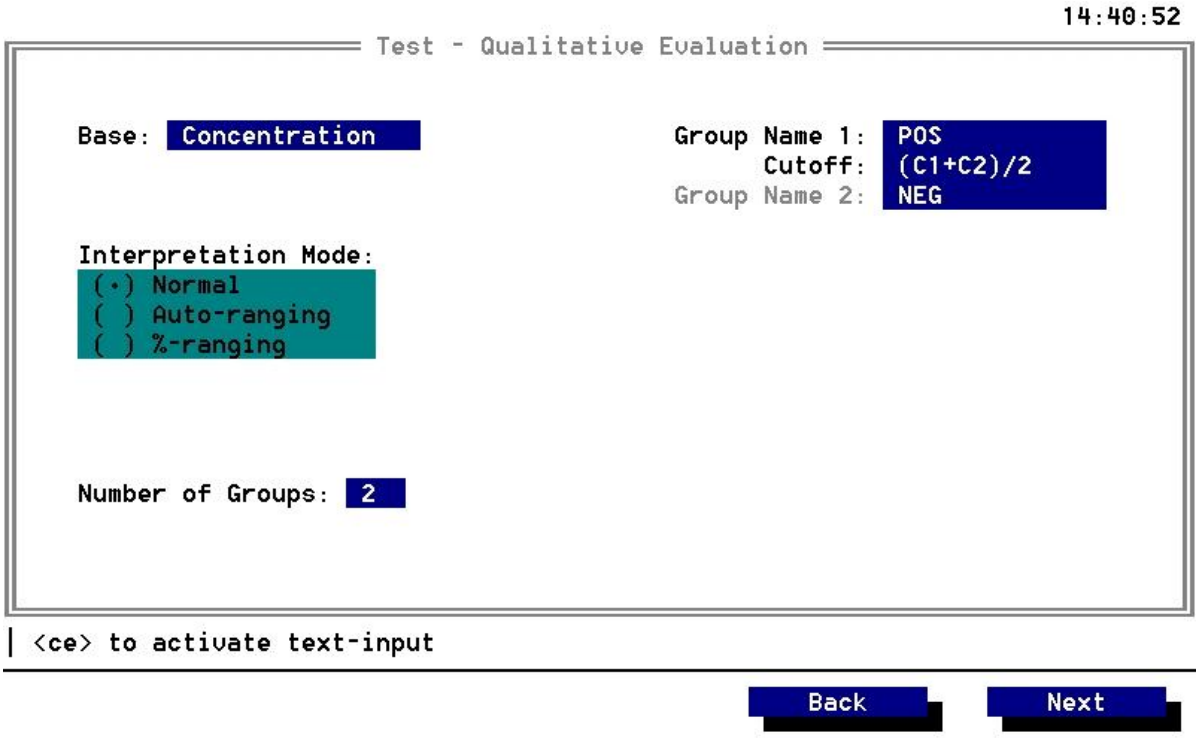
   Definition: "The dose level which results in a response exactly half way between the zero dose response "a" (upper asymptote) and the infinite dose response "d" (lower asymptote) is designated as the "midrange" of the assay and is designated "c". ... In addition, the curve is characterized by a "slope factor" or "exponent", designated by "b"."

   Definition according to "Radio-Immunoassay and Related Procedures in Medicine", International Atomic Energy Agency, Vienna, 1974, Volume 1, p.165 ff.



Drawing 20.: 4-Parameter Fit

6.1.16 **Qualitative Evaluation**



Screen 21.: Qualitative Evaluation

Options:

Base
Select the base values (original measurement values or results of the relative calculation by selecting the 'Result Name' or concentrations) for the qualitative evaluation. If quantitative and qualitative evaluation is combined, the measurement data can also be displayed in concentrations and evaluated qualitatively as such by using the standard curve.

Interpretation Mode
Set the type of threshold value subdivisions

1. **Normal:** Up to four thresholds (cutoffs) can be programmed individually and up to 5 groups selected.

2. **Auto-ranging**: Upper and lower threshold are programmed. The intermediate range can be divided into 2 to 10 equidistant groups.

3. **%-ranging:** Upper and lower limits are programmed as cutoffs. The sample measurement values are printed also as percentages on the printout (the sample measurement values are scaled in percent).

Number of Groups
Available only for “Interpretation Mode: normal”. Select the number of groups to be subdivided (minimum=2, maximum=5).

Number of Divisions
Available only for “Interpretation Mode: Auto-ranging”. Select the number of equidistant tolerance value subdivisions (minimum=2, maximum=10).

6.1.16.1 Cutoffs and Ranges

Cutoff values (CV)
Cutoff values for the results /concentration values / relational values: Enter the formula or constants for the programmed cutoff values (normal: up to 4 cutoffs can be programmed / auto-ranging or %-ranging: two cutoffs must be programmed).

Press **<ce>.** All standards, controls, arithmetic functions and variables, which can be used in the cutoff-formula, appear in the display menu. Enter numbers with the keyboard or select the desired characters with the cursor- and the function-keys. Confirm complete formula by pressing **<enter>.**

Group Name x
All values falling in this group must be within the range defined by the previous and the next cutoff **(CV).** Press **<ce>** to activate the text input and select the desired characters with the up/down cursor keys.

Confirm with **<enter>.** If “**Interpretation Mode: Auto-ranging**” or “**Interpretation Mode: %-ranging**”, the intermediate range between two cutoff values cannot be individually named since either the group or a percentage is automatically printed as the name. This range is referred to as “’**Special**’”.

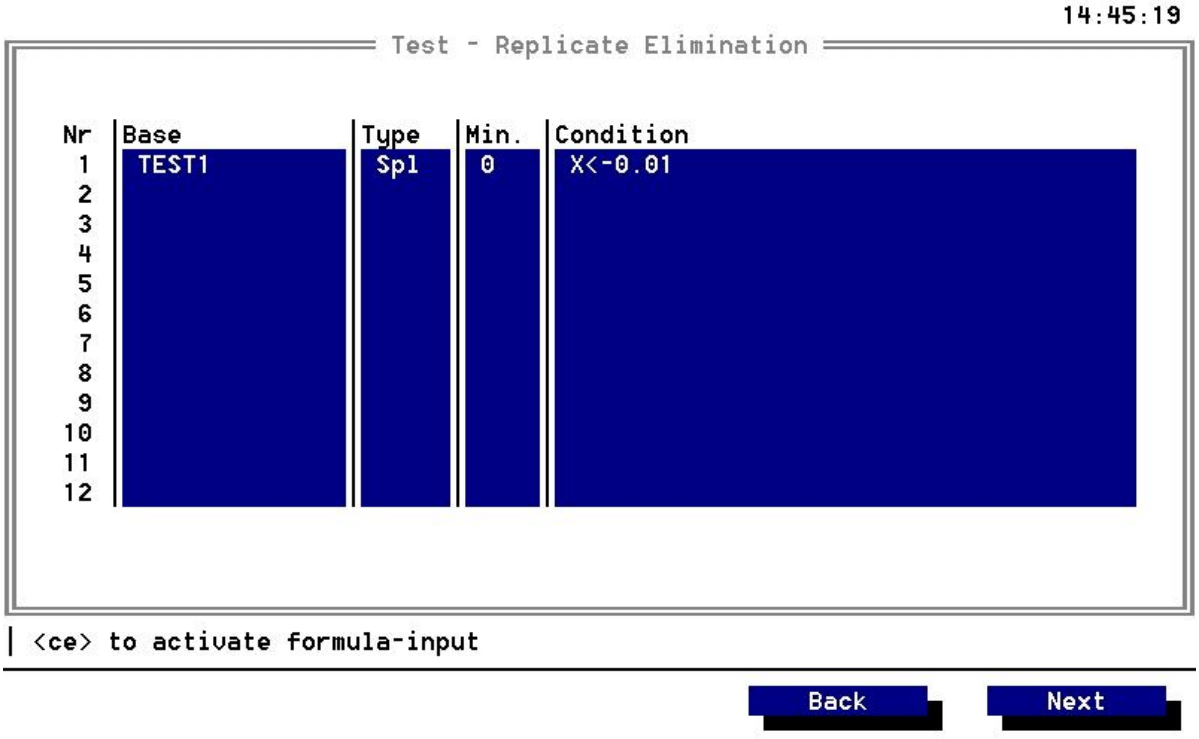
Function keys:

F4 – Back
Return to the previous screen.

F5 - Next
Proceed to the next screen.

6.1.17 **Replicate Elimination**

This function serves to eliminate individual replicates, which do not fulfill a certain user-defined condition. After the first elimination, the mean value of the remaining replicates is re-calculated and the condition is re-examined. If necessary, the elimination cycle is repeated. If a minimum number of replicates (to be defined) are still available in the end, the test is valid, if not, it is marked “invalid” on the printout. If the mean value of the replicates remaining after elimination constitutes the basis for the test evaluation, the necessary conditions are to be defined in the chapter 6.1.18.



Screen 22.: Replicate Elimination

Options:

Column “Base”
Select the base values (original measurement values or results of the relative calculation by selecting the ‘Result Name’ or concentrations) for the calculation for eliminating replicates.

Column “Type”
Definition of the standard / control / blank /sample (Spl) which is to be evaluated with the following condition. Press <ce>, select the corresponding position and confirm with <enter>.

Column “Min.”
Enter the minimum number of remaining replicates (for samples only 0 is possible).

Column “Condition”
Enter one or more conditions, which must be fulfilled by each replicate to avoid elimination. Press **<ce>** to activate the input menu:

All special positions defined in the plate layout (standards, controls, blanks)

Arithmetic functions
(+, -, *, /. (), <, >, =, %)

Logical combinations
(NOT = true if condition is not fulfilled, AND = true if all conditions are fulfilled, OR = true if one or more conditions are fulfilled, XOR = true if only one of the conditions is fulfilled)

Variables (please refer to chapter 6.1.10)
X (represents each individual replicate of the type selected).

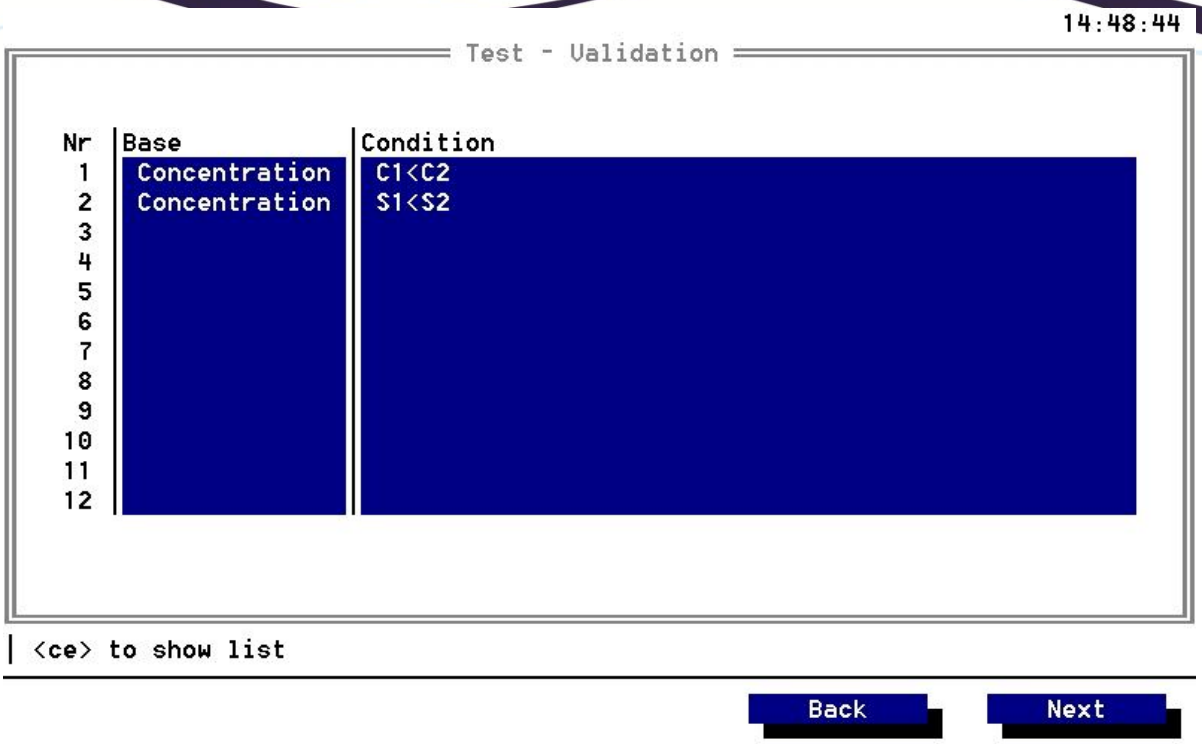
Move cursor to select a row and confirm with the corresponding function key. Delete the last entry with <ce>. Finally re-confirm the formula with <enter>.

Function keys:

F4 – Back
Return to the previous screen.
F5 - Next
Proceed to the next screen.

6.1.18 Validation

If a programmed condition is not fulfilled, the entire test is marked “invalid”.



Screen 23.: Validation

Options:

Column “Base”
Select the base for the calculation.

Column “Condition”
Enter one or more conditions, which must be fulfilled by the entire test to be valid.
Press <ce> to activate the input menu:

All special positions defined in the plate layout (standards, controls, blanks**)**

Arithmetic functions
(+, -, *, /. (), <, >, =, %)

Logical combinations
(NOT = true if condition is not fulfilled, AND = true if all conditions are fulfilled, OR = true if one or more conditions are fulfilled, XOR = true if only one of the conditions is fulfilled)

Variables (please refer to chapter 6.1.10)

Move cursor to select a row and confirm with the corresponding function key. Delete the last entry with **<ce>.** Finally re-confirm the formula with **<enter>.**

Function keys:

F4 – Back

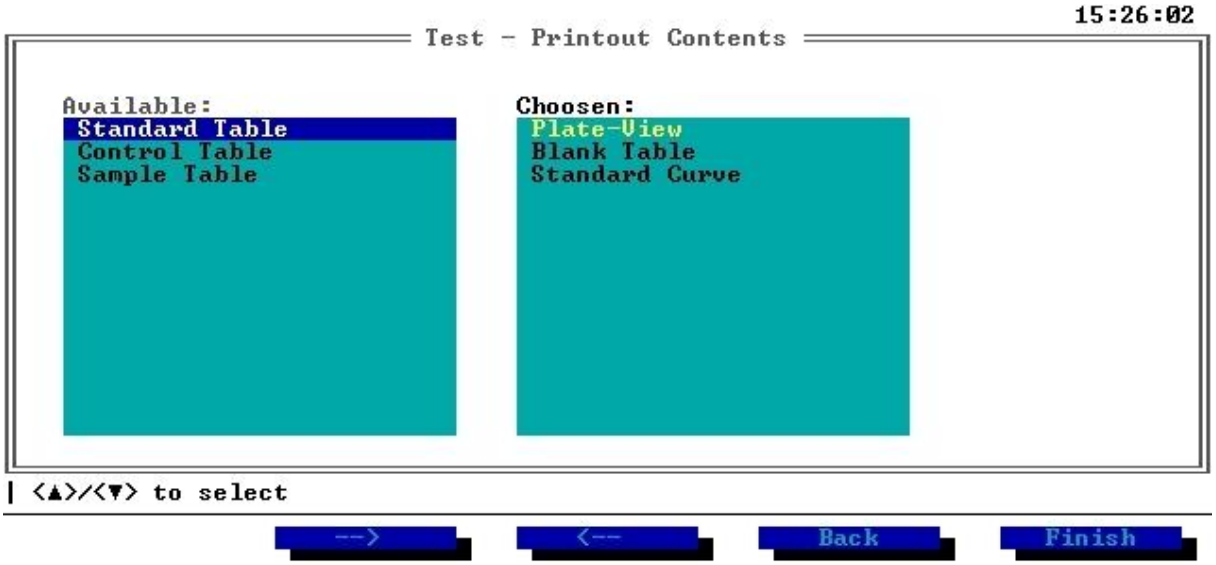
Return to the previous screen.

F5 - Next

Proceed to the next screen.

6.1.19 Printing & Safeguarding the Test in the Test-Memory

6.1.19.1 Printing



Screen 24.: Print Options

Options:

Select the desired printing options in the “Available:” window and move the option into the “Chosen:” window with the function key F2 - “-->”. The options can be removed from the “Chosen” window by selecting and moving the option into the “Available:” window with the function key F3 – “<--“.

Function keys:

F2 – -->

Move the selected printing option from the “Available” into the “Chosen” window.

F3 – <--

Move the selected printing option from the “Chosen” into the “Available” window.

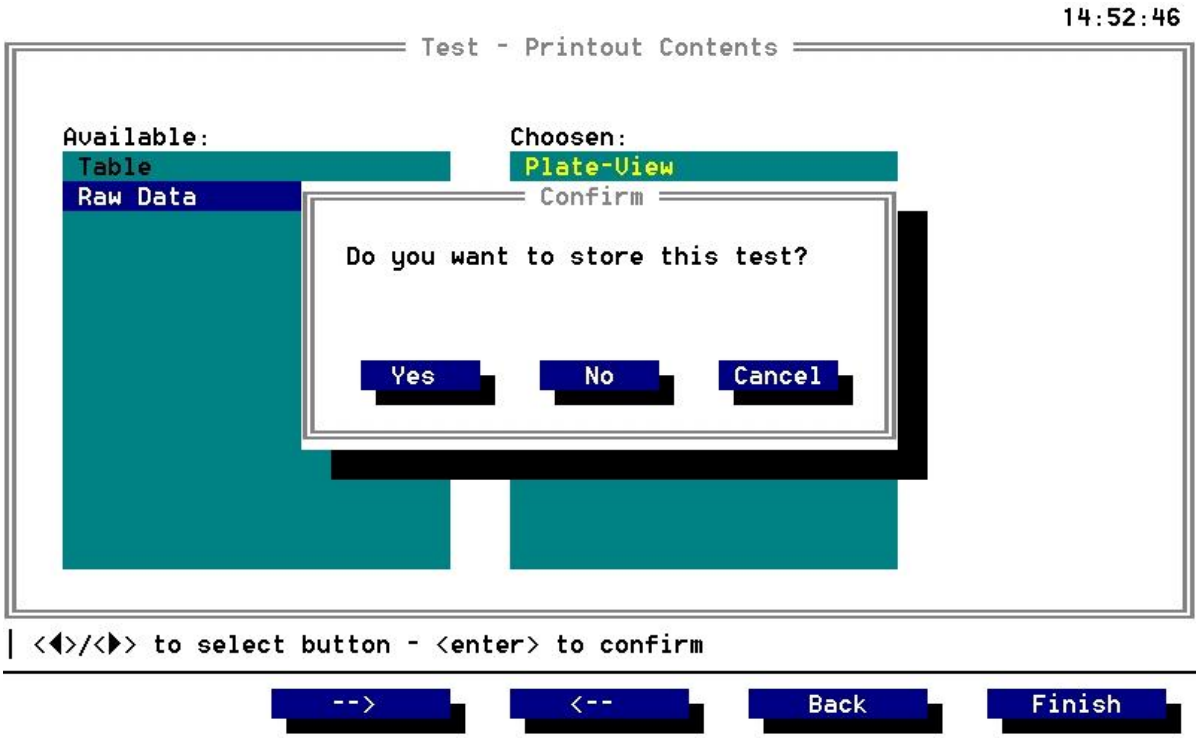
F4 – Back

Return to the previous screen.

F5 - Finish

Finish defining or editing the test and proceed to the storage menu.

6.1.19.2 Safeguarding the Test in the Test Memory



Screen 25.: Safeguarding a test

Options:

Select the options with the cursor keys and confirm the selection with **<enter>.**

Yes

Exit the define/edit mode and store the test in the test memory of the instrument

No

Exit the define/edit mode without storing the test. All definitions/changes will be discarded!

Cancel

Return to the define/edit mode (Finish screen).

Editing and Clearing of Defined Tests

6.1.20 Edit Test – Changing a Test Already Programmed

Press <menu> and select the test menu. Choose the “Edit” option. Select the test which shall be edited in the list and confirm with <ENTER>. Now all parameters of this test can be edited (please refer to chapter 0 for the descriptions of the parameters).

6.1.21 Copy a Test

Follow the steps described above (chapter 6.1.20).

Change the test name in the first screen, make changes to the parameters if necessary and store the test. The test will be stored with the new name. No changes are made to the original test.

6.1.22 Delete Test

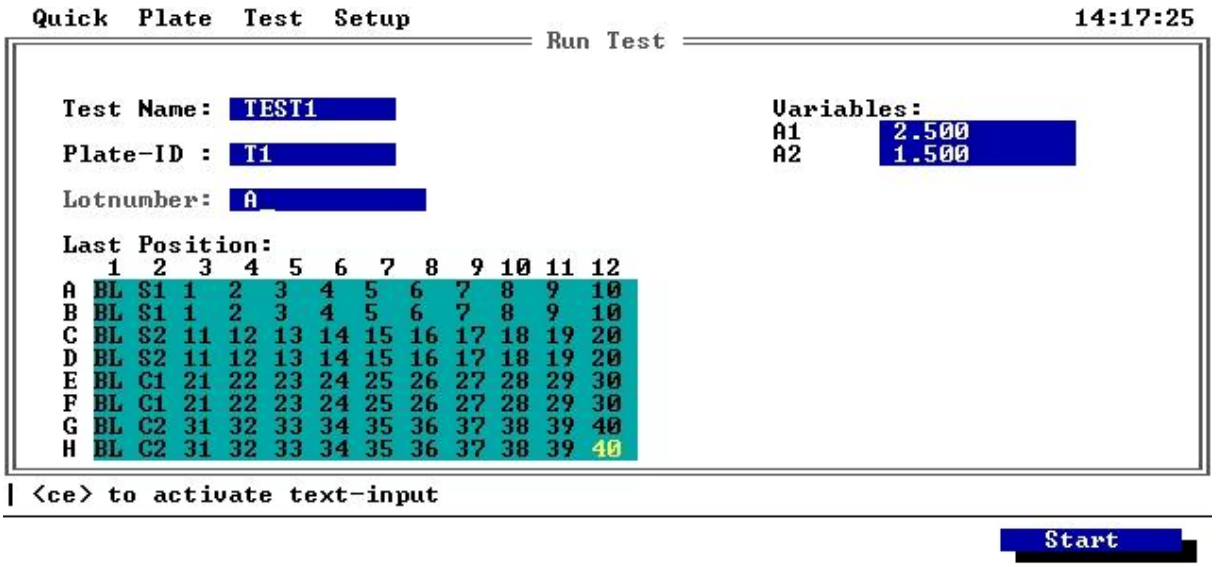
Press **<menu>** and select the test menu. Choose the “**<Delete>”** option.

Select whether a single test or the whole test memory shall be deleted.

Test:
Select the test to be deleted and confirm the selection.

All tests:
Confirm that all tests in the memory shall be deleted.

Run Test



Screen 26.: Run Test

Options:

Test Name
Press **<ce>** and choose the test to be started.

Plate-ID
An automatic plate ID is available in default (YYMMDDxx, incremented from 1 to 99). To enter another plate ID, press **<ce>** to delete the default number, and then scroll up/down for text input. Confirm with <enter>.

Lot-number:
Allows entering a lot number for this plate.

Last Position:
Select the last position with the cursor keys.

Variables:
In case those variables where defined during the test definition, the values of these variables may be changed prior to run the test.

Function keys:

F5 – Start

Start the selected test with the above entered plate-ID

The progress of the test is displayed in the “Test State” window.

As soon as the test is measured and calculated, confirm the “Test State” window with <enter>. The measurement results are displayed.

Measurement Results

The measurement results are displayed immediately after the measurement was performed.

To re-view the results of a measured plate, select “View” in the plate menu. Choose the plate to be re-viewed and confirm with enter; – please refer also to chapter 6.1.32).

The first screen, which is displayed, is the “Test State” screen. This screen shows the information concerning the evaluation of the plate.

To return to the main menu, press <stop> twice.

Function keys:

The function keys <F1> to <F4> have different functions, depending on the data displayed on the screen.

F5 – Sel. Screen
Depending on the performed measurement, different screens are available:

6.1.23 Info Screen

The screen appearing first after each measurement or re-evaluation of data is the info screen, showing the plate name, test name, lot number, measurement date and time and the variables (only if variables were defined for this test).

If the evaluation of the test run was invalid, a list of error messages is displayed.

Function keys:

F1 – Print

Prints information concerning the test and measurement time. Also, the results are printed according to the settings in the test definition (please refer to chapter 6.1.19.1). Make sure a printer is connected and on-line, and that the correct printer is selected in the system-setup.

F2 – Save As

Appears only if a plate had been re-evaluated. This function enables to store the plate with a new name.

F5 – Select Screen

6.1.24 Raw Data Screen

This screen is only displayed for measurements, where raw data are available (e.g. kinetic measurement) results. In this screen the measured values for every well are displayed.

Function keys:

F1 – Well-Graph

Displays the reaction curve of the selected well.

F2 – Plate-Graph

Displays the reaction curves of all well of the plate.

F3 – Prev RawDat

Displays the raw data of the previous measurement.

F4 – Next RawDat

Displays the raw data of the next measurement.

F5 – Select Screen

6.1.25 Measurement Result Screen

Displays the measured values in the plate layout.

Function keys:

F5 – Select Screen

6.1.26 Plate Layout Screen

In this screen the plate layout is displayed. Additionally certain wells can be rejected (R – to be set/unset with the **<ce>** key). This option is meant to be able to reject so-called “outliers” and to be able to re-calculate the plate without these wells. If all replicates of one standard are deleted, the plate can be re-calculated without this standard.

Function keys:

F1 – Re-Calc
To re-calculate the plate in case that the rejection state of one ore more wells had been changed.
F5 – Select Screen

6.1.27 Relational Result Screen

Is referred to under the name given in the test definition for the relational results.

The recalculated (with the formula defined in the test parameters for relative calculation) results are displayed on the screen.

Function keys:

F5 – Select Screen

6.1.28 Quantitative Results Screen

This screen is only available, if quantitative calculation was defined in the test parameters. It shows the results of the quantitative calculation. The name of this screen will change to the concentration unit name (e.g. MG/ML), which was set up in the test definition!

Function keys:

F5 – Select Screen

6.1.29 Standard-Data Screen

This screen is only available, if quantitative calculation was defined in the test parameters. It shows the data of the standard curve.

Function keys:

F1 – Re-Calc
To re-calculate the plate in case that curve parameters had been changed.

F3 – Graphics
Displays the standard curve according to the defined parameters.

F5 – Select Screen

6.1.30 Qualitative Results Screen

This screen is only available, if qualitative calculation was defined in the test parameters. It shows the results of the qualitative calculation.

Function keys:

F5 – Select Screen

6.1.31 CV % Screen

This screen shows the percentage correlation of variation of replicate standards, controls and samples. The value is shown on the upper left plate position of replicates.

Function keys:

F5 – Select Screen

Plate Data Storage

After each measurement with a pre-programmed test, all measurement and evaluation data, all test parameters and the plate identification number are stored in the plate memory. If the memory is full, the respective error message will be displayed on the screen.

The plate identification number (combination of letters also possible) is entered prior to the measurement in the “Run test” menu.

6.1.32 View Plate

To re-view the measurement results of a certain plate, select the plate as described above.

Press <menu>, select “Plate”, then “View” and choose the desired plate. Now the result screen is activated. For details concerning the measurement results, please refer to chapter 0.

6.1.33 Print Plate

Press <menu>, select “Plate” and then “Print”. Select the plate to be printed. Now all results are printed. Make sure a printer is connected and on-line, and that the correct printer is selected in the system-setup.

6.1.34 Re-Evaluate Plate

Press **<menu>,** select **“Plate**” and then **“Re-Eval.”.** Select the plate of which the measurement values shall be re-evaluated. Select the test with which the plate shall be re-evaluated.

Now the selected plate is re-evaluated with the currently selected test. For detailed information concerning the results, please refer to chapter 0.

6.1.35 Delete Plate

To delete a plate, please select this plate as described above.

Press <menu>, select “Plate” and then “Delete”. Either the selected plate (“Plate”) or the whole plate storage (“All Plates”) can be deleted. Re-confirm your selection with “Yes” and <enter> or discard it by selecting “No” and <enter>.

***Please Note**:* Please make sure to have printouts of the result available prior to deleting it!

6.1.36 Floppy Disk Data Management

6.1.36.1 Save a Test to Disk

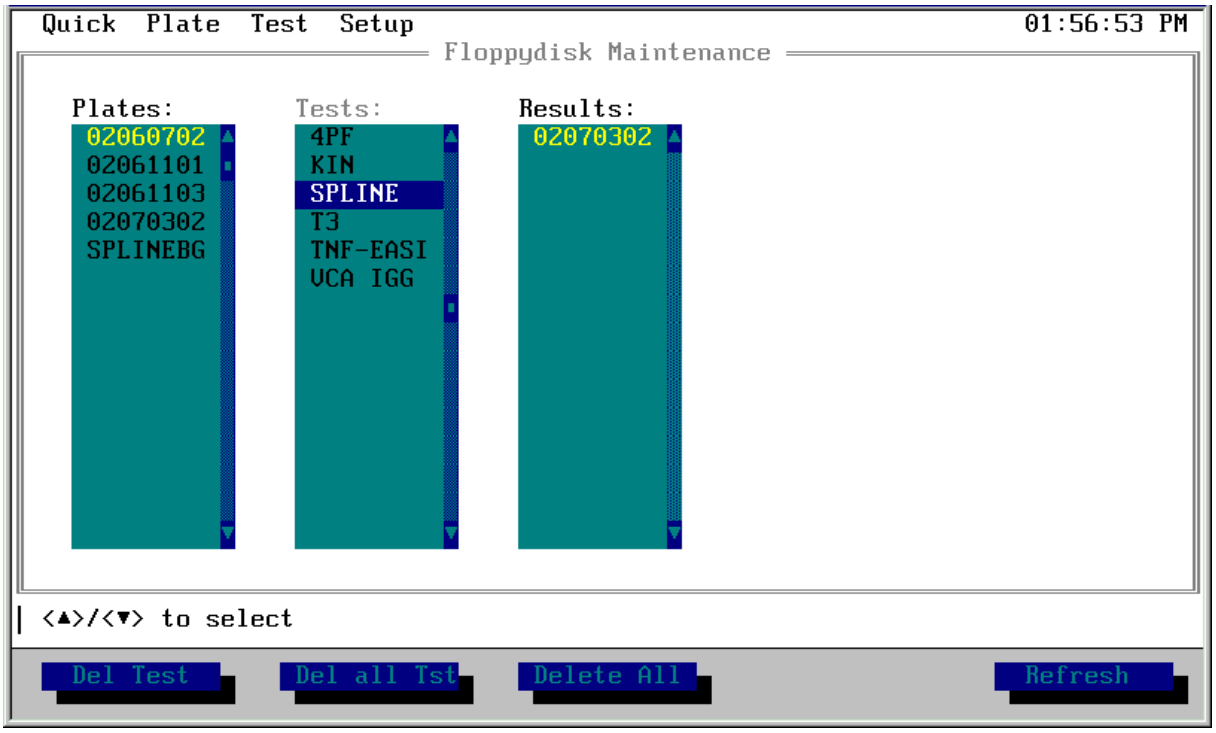
Press <menu> and select the test menu. Choose the “**store**” option. Now “**copy test**” or “**copy all tests**” (or "**shift test**" or "**shift all tests"**) can be selected.

Copy: The copy option copies the data from the Anthos 2020 to the disk; the data is now available on both, the instrument and the disk.

Shift: The shift option moves the data from the storage of the Anthos 2020 to the disk; the data is now available only on the disk.

Select “copy test” and confirm with **<ENTER>** to copy a single test to disk, select “copy all tests” to store all tests to disk. Now select the test to be stored to disk and press **<ENTER>.**

6.1.36.2 Delete a Test from Disk



Screen 27.: Floppy disk Maintenance

Press <menu> and select "Floppy Disk Maintenance" in the "setup" menu. Select (highlight) the data you like to delete and press one of the following:

Function keys:

F1 – Del Plate (Del Test / Del Result)
Deletes the highlighted data (plate, test or result)

F2 – Del all Plt (Del all Tst / Del all Res)
Deletes all data in the selected (highlighted) list (plates, tests or results)

F3 – Delete All
Deletes all data (all plates, tests and results) on the floppy disk.

F5 - Refresh
Re-reads the data stored on the inserted floppy disk.

6.1.36.3 Retrieve a Test from Disk

Press **<menu>** and select the test **<menu>.** Choose the "**retrieve**" option and press **<ENTER>.** Choose single "**test**" or "**all tests**" and confirm with **<ENTER>.** Now select the test to be retrieved and confirm with enter.

6.1.36.4 Export Plate Data as Text File

Press **<menu>** and select the plate **<menu>.** Choose the "**Export Text File"** option and press **<ENTER>.** Choose single **"plate"** or **"all plates"** and confirm with **<ENTER>.** Now select the plate to be exported and confirm with **<enter>.**

7 Maintenance

Approved parts

Except for the parts shown in the following list, only parts supplied by Anthos or an authorized Anthos Distributor may be installed in or used with the Anthos 2010:

Fuses: as specified in chapter: **3.1.3 Rated operating conditions**

Power cable: CE-marked power cable with connected protective earth and protective earth connector (see also **0**)

External Computer controlling the instrument
(see also **0** Connecting 2020 with an external computer)

Cleaning and Disinfecting the Instrument

Clean the reader regularly and immediately after spillage. This has to be done with due care and attention. Always observe laboratory safety rules and regulations. Do not use force when cleaning the reader.

For disinfection, please follow the procedure below. Authorized trained personnel in a well-ventilated room while wearing disposable gloves, protective glasses and clothing should perform the cleaning and disinfection procedures.

The instrument should be

Please Note: Before the reader can be returned to base for service, it must be disinfected and a disinfection certificate must be completed.

1. Switch off the reader and disconnect it from the mains power supply and the PC.

2. Carefully wipe off the entire reader with lint-free tissues that have been moistened in a protein degrading mild detergent or a saline solution.

3. Carefully wipe off the entire reader with non-lint tissues that have been moistened in a 70% ethanol or a 0.5% bleach solution.

4. Soak non-lint tissues that have been moistened in a 70% ethanol or a 0.5% bleach solution onto the plate transport mechanism and let it soak for ± 30 minutes.

5. If a bleach solution has been used, carefully wipe off the entire reader with non-lint tissues that have been soaked in water.

6. Dry the reader by wiping it off with non-lint tissues.

Changing a Fuse

In case of malfunction, the fuses (in the mains filter next to the mains socket on the rear of the device) can be checked and replaced.

1. Disconnect the instrument from mains by unplugging the power cable.

2. Open fuse-carrier next to the mains socket with a screwdriver

3. Remove fuse

4. Insert spare fuse included in supply and specified earlier in this manual

5. Close fuse-carrier.

6. Turn device on and check functioning. In case of malfunction, call a service technician.

Error Messages

7.1.1 Mathematical Error Messages

A

Addition: is displayed if the argument left or right of the + is missing.**Argument not in domain of function** (Undefined result): is displayed when an Argument of a formula is out of the defined range of this operation (e.g. Log (-1)).

C

Calculation of well XNN: (state window) - is displayed if the calculation of the transformation is erroneous.

Correlation too low: (state window) - is displayed if the quantitative evaluation is erroneous.

Cut-Off Values: (state window) - is displayed if the qualitative evaluation is erroneous (e.g.: calculation of the cut-off values; cut-off values are not increasing).

D

Division: is displayed if the argument left or right of the / is missing.

Division by zero: is displayed when a mathematical operation results in a division by zero.

F

Formula Error: (state window) - is displayed if the evaluation formula is erroneous (e.g. replicate elimination; calculation of the test validation).

Function: e: is displayed if the argument of the Exp function is missing.

Function: ln: is displayed if the argument of the Ln function is missing.

Function: log (base 10): is displayed if the argument of the Log function is missing.

Function: power: is displayed if one or both arguments of the Pow function are missing.

I

Incorrect number-format: is displayed when a number with more than one comma is entered (e.g. 12.34.5).

Inversion: is displayed when the argument after NOT is missing (e.g. NOT()).

L

Left parenthesis expected: is displayed when the left parenthesis of a formula is missing.

M

Mathematical error: (state window) - is displayed if the quantitative evaluation is erroneous (e.g. linear regression; 4-paramter-fit).

Multiplication: is displayed if the argument left or right of the * is missing.

N

Number length exceeded: is displayed when number is longer the 14 characters..

O

Overflow: is displayed when the result of a mathematical operation exceeds the maximum of the positive range (e.g. Exp (1000)).

P

Parameter expected: XXX: is displayed if the argument left or right of an operator is missing (e.g.: 1>).

Precondition: is displayed when the result of a mathematical operation is out of the specified range (e.g. the response value for a 4-parameter-fit is less or equal the lower asymptote).

R

Result: is displayed if the entered formula gives more or less the one result.

Result is a singularity: is displayed when the result of a mathematical operation would be a singularity (e.g. Pow (0, -2)).

Right parenthesis expected: is displayed when the right parenthesis of a formula is missing.

S

Spline: (state window) - is displayed if the quantitative evaluation is erroneous (e.g. curve not definite).

Standard-Point Calculation: (state window) - is displayed if the quantitative evaluation is erroneous (e.g.: calculation; monotony).

Subtraction: is displayed if the argument left or right of the - is missing.

T

Total loss of significance: is displayed when the digits behind the comma are not correct.

Type mismatch: is displayed when the entered formula is not correct (e.g.: (X>0.5)AND(1))

U

Underflow: is displayed when the result of a mathematical operation exceeds the maximum of the negative range (e.g. Log (-1)).

Unknown character: X: is displayed when the formula contains an unknown character (e.g.: X*~); this is only possible, when the instrument is controlled with an external PC

V

Variable not found: is displayed when formula contains a variable, which is not defined.

7.1.2 Device and Communication Error messages

sb1.1 FILTER ERROR

If the fw value in NORMAL MEASUREMENT command (reefer chapter 10.7) does not match the fw from the Anthos 2020 lists, the FILTER ERROR flag

and ALL ERROR flag are set. This is the information that the requested filter is not installed in the Anthos 2020. Also a movement of the filter wheel during a NORMAL MEASUREMENT command or an error in the SET FILTER command can cause this error.

sb1.2 TRANSPORT INIT ERROR

The error may occur during the transport initialization and is reported through this status flag. It indicates that the transport system is not in the load position. A defective motor, transport mechanism or sensor could be possible reasons for this error.

sb1.3 PRINTER BUSY ERROR

Before a byte is transmitted to the printer the BUSY line is checked. A 0.5 seconds timeout is set for waiting for the printer. Possible reasons: printer is OFF-LINE, no paper.

sb1.4 PRINTER ACKNOWLEDGE ERROR

Indicates no ACK response from printer for transmitted characters. Only the GET PRINTER STATUS command checks the ACK signal. A switched-off printer can be the reason for this error!

sb1.5 AD TIMEOUT ERROR

Means that there is no signal from ADC Chip. This indicates a hardware error.

sb1.6 LAMP ERROR

The MAX. measurement is done with diode values between 60 000 to 65 000. If it is not possible to reach this lamp energy level the LAMP ERROR is reported. Possible reasons:

1. The lamp, lamp connections and/or the lamp regulation are defected.
2. The transport system is not in the load position.
3. The filter wheel is not in the correct position.
4. The lamp energy value is not correct (an error, which may have been coming up during the SET FILTER command, or an EEPROM error).

sb1.7 Y SENSOR INTERRUPT ERROR

The movement of the transport system is not correct (i.e. the location of the wells is not correctly in the optical path).

sb2.0 MEASUREMENT TIMEOUT ERROR

The motor of the transport system does not move or a transport sensor is defected.

sb2.1 Watch-Dog ERROR

Set by the Watchdog reset routine. Indicates an unknown, usually undetermined system error.

sb2.2 TO LOW MOTOR SPEED ERROR

The transport speed is too slow.

sb2.3 DELTA MAX TO HIGH

The lamp energy is not stable.

sb2.4 START POSITION ERROR

The transport system is not in the load position. The transport INITIALIZE command should be repeated!

sb2.7 ALL ERROR

This is a common error status bit which is logical OR of all the individual error status flags. This is a read only status bit useful for a quick check if any error status flag is set. The GET STATUS command clears this and other error status bits.

8 Appendix:

Principles of Photometry Measurement

Light is electromagnetic wave radiation. Rays from 100 nm to 400 nm are defined as the ultraviolet spectrum of light. Only rays in the range from 400 nm to 780 nm are visible for the human's eye, rays with longer wavelengths are called infrared. Color impressions are caused by reflection of electromagnetic waves striking the surface of material substances. Substances absorb the complementary spectrum of their visual perceptible color. Hence green plants look green since they absorb red light (light of a wavelength perceived as red, e.g.: approximately 750 nm). A photometer is an optoelectronic measuring device to determine the amount of light absorbed at a specific wavelength. Therefore a photometer can be used to determine the concentration of a certain light-absorbing substance in a colored solution.

8.1.1 Absorbency reading

Experimental measurements return the value of transmission (**T**):
Transmission is defined as the percentage ratio between the total available light energy at the detector (measured through air) and the residual luminous energy after sample transmission at a specific wavelength.

$$\mathbf{T = I/I_0}$$ (I.....Light intensity after passing through the sample

I_0.... Initial light intensity)

Transmission has no linear relation to concentration; therefore **A**bsorbency (also called **O**ptical **D**ensity) is calculated from Transmission by the formula:

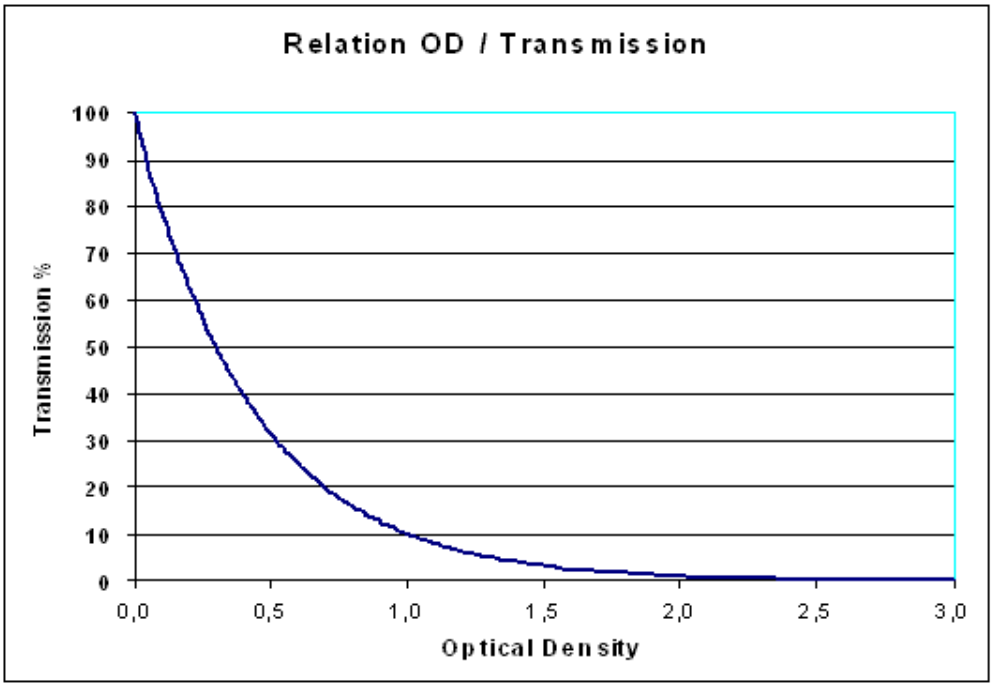
$$\mathbf{A = OD = -\log T}, \text{ or } \mathbf{A = OD = \log (1/T)}$$

Transmission is not related to layer thickness (path-length of the light) and sample concentration in a linear way:
If one layer of a certain material allows 10% transmission, doubling either path-length or concentration would bring down the transmission to 1% and doubling both would bring it down to only 0.01% (logarithmic relation)!
Absorbenceis related in a linear way to path length and sample concentration as is described in Beer Lambert's Law:

Absorbance (OD) = c * d * e

c = the concentration of the sample to be measured
d = the layer thickness of the sample to be measured
e = the molar extinction coefficient

The following graph illustrates the relation between Absorbency (sample concentration) and the light transmitted and measured at the detector:



It is important to keep in mind that the best measurement range of Absorbency is from 0.1 to 1 OD or 90% to 10% of transmission. Measurements above 2 OD deal with less than 1% of the original light and will therefore have lower resolution and accuracy.

8.1.2 Measurement at Specific Wavelengths

Each substance to be measured has a specific transmission profile that indicates its concentration and composition. A measurement with white light renders different sample concentrations in a certain spectral range only to a small amount and accordingly inaccurate. A higher significance can be obtained by using only that part of the light spectrum, which is relevant to prove the respective wavelength of the substance.

Due to this fact interference filters of a narrow banded wavelength spectrum are employed in this instrument. Each substance has a certain absorption spectrum. A measurement should be performed by selecting the correct filter for the maximum absorption of the sample, because in this way the best differentiability of various sample concentrations can be reached. We can also state that the differing absorption amplitude of two uniformly composed samples is a measure for the concentration. For this reason measurements in the flank area of the spectrum are not very accurate and therefore to be avoided. In this connection, it has to be taken into consideration that - when using interference filters – wavelength tolerances of +/- 2 nm from the nominal wavelength value may also result in inaccuracies. Each sample has an absorption minimum not specific to the measurement value, which can be deducted from the measurement value automatically by choosing a suitable reference wavelength. This kind of measurement is referred to as bichromatic.

Evaluation applications:

8.1.3 Quick reference for test programming:

8.1.3.1 Bichromatic reading:

It is highly recommended to use a reference reading for subtracting unspecific signals.

Typical measurement and reference filters are:

Substrate:	Measurement Filter:	Reference Filter:
PNPP	405 nm	620 nm
TMB	450 nm	620 nm
OPD	492 nm	620 nm
ABTS	405 (414) nm	492 nm

8.1.3.2 Blank against Air:

An automatic self-calibration (determination of 100% and 0% transmission signals) is performed by the reader for each measurement channel and each particular filter previous to readings. This is equivalent to the "blanking against air", which is often mentioned in kit instructions.

8.1.3.3 Plate Layout:

Singles or replicates of blanks, standards, controls and samples may be distributed freely across the plate. There are no fixed positions used for calculations. The software will find the right wells according to the set names in the plate layout. The mean value is calculated automatically for all replicates of a blank, control, standard or sample.

8.1.3.4 Blank Subtraction:

The mean of positions marked as B (blank) is subtracted automatically from all other wells. If a blank should not be subtracted, it has to be named as a control.

8.1.3.5 Single Point Calibration, Index Calculation, B/B_0 Calculation:

The Transformation Formula allows calculating a ratio to a particular control for all other controls, standards and samples. A common application is the calculation of the percentage of binding with competitive assays (e.g. X‘ = X/C1*100, where C1 is the maximum binding control). With the use of additional concentration factors and correction factors, this formula provides also single point calibration and cut-off ratios. On the printout the result of this formula is reported with its given name.

8.1.3.6 Use of Variables:

Lot dependent values, such as calibrator concentrations or correction factors, may be programmed as variables. Enter default values for the number of variables required. During programming you can use the names V1, V2, ..., which refer to the list of defined variables. At the beginning of each measurement the variables are displayed for review. At this time permanent changes can be made, so that the values are kept according to current kit lots.

8.1.3.7 Quantitative Evaluation:

Concentration values are calculated by means of the calibration curve.
Linear regression is specifically applicable, if the calibration curve is really expected to be straight on a linear or logarithmic concentration axis.
Point to Point and Cubic Spline curve fits are generally suited for non-linear curves, which are not completely straight but slightly bent. However, the Cubic Spline will not tolerate big jumps and turning points and will show a tendency to swing out at such occasions. Point to Point may not be that accurate, but is completely save and robust under all conditions. For both the concentration axis has to be selected linear or logarithmic, according to the sequence of standard points:

The 4-parameter Fit is a non-linear regression, especially suited for competitive assay formats. Its theory assumes an inflection point and two asymptotes at the extreme ends. The axis division is fixed to in/log. Zero concentration standards must not be included in the curve, but in case of competitive assays they should be named as negative controls (e.g. NC1) and may serve for the calculation of B/B_0 by means of the relational formula (X/NC1*100).

The 4-parameter fit is also generally suited for non-linear curves with sandwich ELISA assays. If a turning point is not present, it will adapt to the lower or upper branch of a sigmoid curve. It is more stable than the Cubic Spline and will never overshoot, but may need slightly longer calculation times.

Extrapolation, set in %, allows calculating sample concentration also in a small area below and above the extreme standards. Extrapolated results are marked on the printout with an "X". Results out of range are shown with the prefix > or < to the highest or lowest valid concentration (border of extrapolated area). A few percent extrapolations are recommended, in order to get both replicates of extreme standards into the valid area for results (otherwise the mean of replicates is the borderline).

8.1.3.8 Set-up of Standards and Axis Division:

Standards have to be entered in increasing order of concentrations always (lowest concentration S1). In case of competitive assays, this will produce a falling curve.

If the standard series is produced by a more or less constant adding (e.g. 10; 20; 30... or even 10; 20; 40; 70...), the axis division is linear. If there is a multiplication (dilution) factor (e.g. 2; 4; 8; 16;.., or 10; 33; 100; 330;...), the axis division is logarithmic.
Zero concentration standards must not be used on a logarithmic scale for mathematical reasons. If such a standard sequence is suggested in the kit instructions, do not use the zero standard for the curve (recommended) or set it to a small positive value, two logs below the second standard (e.g. 0.02; 2; 4; 8;...)
The identifiers for standards on the plate (S1 – SXL) may be replaced by fixed OD-values or formulas. This allows using stored standard curves with correction factors (refer to 8.1.4, Storing a Standard Curve).

8.1.3.9 Use of Interpretation:

The "Interpretation" section provides not only cut-off calculation based on optical density. If the calculation base is set to the result name of the Transformation or to Concentrations, also these results may be grouped by means of the cut-off formulas. Free names can be given to these groups (up to five).

8.1.3.10 Validation:

The validation formulas describe true conditions on basis of optical density values.
A bold warning will be stated on the printout, if one of the validation criteria's is not fulfilled.

8.1.4 Storing a Standard Curve

The use of stored standard curves is not generally recommended or applicable for the quantitative evaluation of ELISA's. It is explicitly the responsibility of the user to take advantage of the described software possibilities in the appropriate way!
Once a standard curve has been measured and OD results have been obtained (by programming for standard S1 the response value as S1, for

the standard S2 as S2, etc.), the curve can be copied to another test procedure the in the following way:

- Make a printout of the measured plate with standards, containing both concentration values and OD values.
- Copy the original test procedure to a new file by changing the name and editing the test (a copy will be stored under the new name).
- Put in the OD-values of the measured standards S1 – Sx as default values for Variables V1 – Vx
- The layout does not need to contain a real standard anymore. Fill the plate just with samples
- As response values enter the variables V1 – Vx instead of the standards S1- Sx .

When starting a reading with the new test, the variables are displayed and may be updated to the last reading of a standard series.
In order to correct plate-to-plate variations of the assay, it is highly recommended to have at least one real standard on the plate by which a correction factor is calculated. The correction factor is the actual measurement value divided by the initial measurement value of the repeated standard.

Example: A plate with 5 standards is programmed:

Standard Points	S1	S2	S3	S4	S5
Concentration Values	0.1	1	10	100	1000
Response Formulas	S1	S2	S3	S4	S5

A new test is programmed: The measured OD's of the standards are entered to the variables:

Standard Points	S1	S2	S3	S4	S5
Response Values [OD]	0.3	0.6	0.8	0.9	1.0
Put to Variables	V1	V2	V3	V4	V5

Only standard S3 is used on the plate-layout of the new test. It is named C1 (control) and its measured value is used to calculate the correction factor for all other standards:

Standard Points	S1	S2	S3	S4	S5
Concentration Values	0.1	1	10	100	1000
Response Formulas	V1*(C1/V3)	V2*(C1/V3)	V3*(C1/V3)	V4*(C1/V3)	V5*(C1/V3)

If the actual measurement value of the standard 3 (C1) deviates from the original measurement value (V3), all standards are re-adjusted by the correction factor C1/V3 corrected:

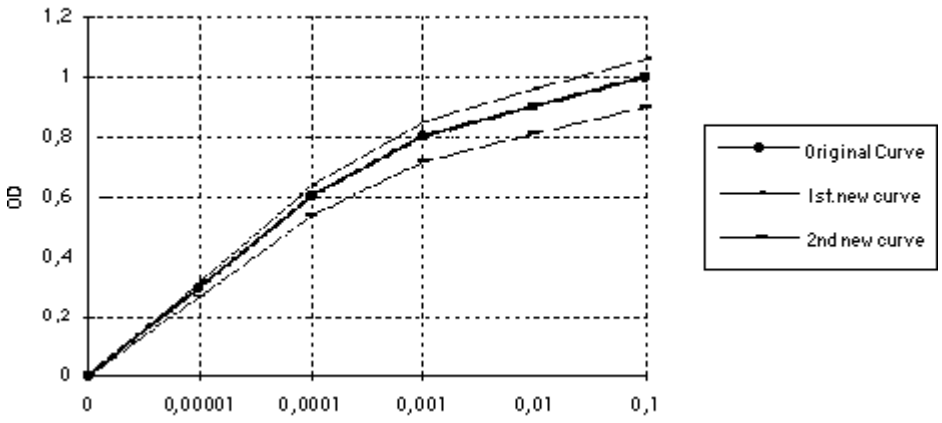


Fig. 5: Standard Curve Graph

8.1.5 Kinetic reading for extended range ELISA's:

With photometry endpoint readings, the logarithmic scale of optical density may be a limiting factor for some ELISA applications. Quantification of TSH, for instance, would benefit from a broader dynamic range than normally available.

If the substrate is TMB, kinetic reading can extend the measurement range of the assay. The result of the kinetic data reduction (e.g. mOD/min) may be used as the basis for any further quantitative and/or qualitative evaluation.
The maximum slope (maximum reaction speed) of the color development at 620 nm corresponds to the amount of bound enzyme. (In fact, stopping of the color reaction means setting of an *artificial endpoint* and uses also the different reaction speed of different enzyme concentrations).

Example of Test Parameters:

Measurement filter:	620 nm
Interval time:	20 seconds
Number of readings:	30 up to 40
Interval shaking:	3 - 5 seconds, medium speed
Data reduction:	Maximum slope

The total kinetic measurement time can be set shorter than the normal substrate incubation time. TMB measured in the „blue phase“ at 620nm has about one third of the optical density than the same concentration measured after stopping in the „yellow phase“. Samples and standards, which would reach "overflow" in an endpoint reading, will remain within the good measurement range during kinetic readings. Therefore the dynamic range and accuracy of ELISA‘s may be significantly improved with kinetic readings.

A main advantage of kinetic readings is the elimination of the critical timing between adding of substrate and stopping solution for all wells.