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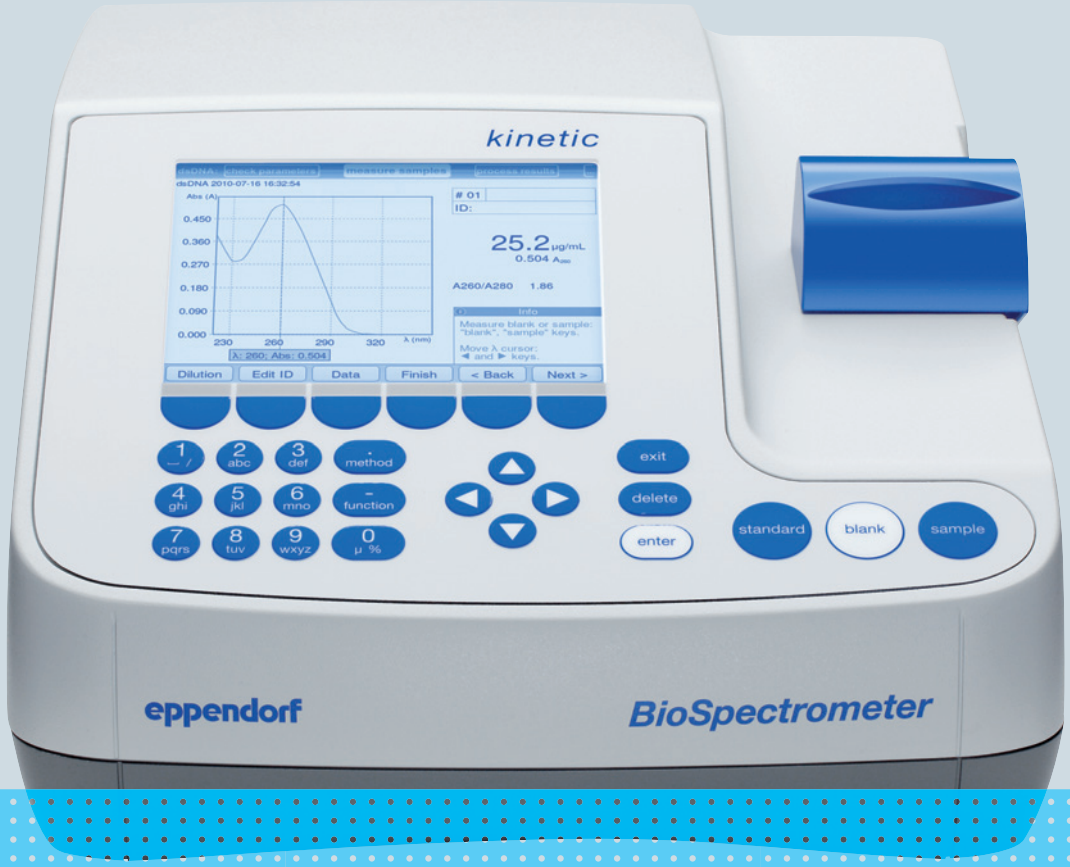

Eppendorf BioSpectrometer® kinetic

Operating manual

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1 Operating instructions

1.1 Using this manual

- Read this operating manual completely before using the device for the first time. Also observe the instructions for use of the accessories.
- This operating manual is part of the product. Thus, it must always be easily accessible.
- Enclose this operating manual when transferring the device to third parties.
- You will find the current version of the operating manual for all available languages on our webpage under www.eppendorf.com.

1.2 Danger symbols and danger levels

The safety instructions of this operating manual indicate the following danger symbols and danger levels:

1.2.1 Danger symbols

	Electric shock		**Risk of explosion**
	Toxic substances		**Hazard point**
	Material damage		

1.2.2 Danger levels

DANGER	*Will* lead to severe injuries or death.
WARNING	*May* lead to severe injuries or death.
CAUTION	May lead to light to moderate injuries.
NOTICE	May lead to material damage.

1.3 Symbols used

Depiction	Meaning
1. 2.	Actions in the specified order
▸	Actions without a specified order
•	List
 or **sample**	Press this key to perform the described action.
 or [Copy]	Press this softkey to perform the described action.
	Additional information

1.4 Abbreviations used

A
Absorbance

DNA
Deoxyribonucleic acid

dsDNA
Double-stranded DNA

Dye methods
Methods for **Dye labels** group for measuring dyed biomolecules

FOI
Frequency of Incorporation: Measure for the amount of dye molecules in relation to the amount of nucleotides in dyed biomolecules

M
mol/L (*molar*)

OD600
Optical density at a wavelength of 600 nm

RNA
Ribonucleic acid

ssDNA
Single-stranded DNA

UV
Ultraviolet radiation

Vis
Visible light

CV
Coefficient of variation (standard deviation/average value) in percent

2 Safety

2.1 Intended use

The BioSpectrometer kinetic is to be used in molecular biology, biochemistry and cell biology research laboratories. The BioSpectrometer kinetic is exclusively intended for use indoors. All country-specific safety requirements for operating electrical equipment in the laboratory must be observed.

The BioSpectrometer kinetic is used for photometric concentration determination of analytes in liquids and recording of absorbance wavelength spectra in cuvettes.

Only use Eppendorf accessories or accessories recommended by Eppendorf.

2.2 User profile

The device and accessories may only be operated by trained and skilled personnel.

Before using the device, read the operating manual carefully and familiarize yourself with the device's mode of operation.

2.3 Warnings for intended use

2.3.1 Personal injury

DANGER! Electric shock as a result of penetration of liquid.

- Switch off the device and disconnect the power plug before starting cleaning or disinfection work.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not spray clean/spray disinfect the housing.
- Only plug the device back in if it is completely dry, both inside and outside.

DANGER! Risk of explosion.

- Do not operate the device in areas where work is completed with explosive substances.
- Do not use this device to process any explosive or highly reactive substances.
- Do not use this device for processing any substances which could generate an explosive atmosphere.

WARNING! Electric shock due to damage to device or mains cable.

- Only switch on the device if the device and mains cable are undamaged.
- Only use devices that have been properly installed or repaired.
- In case of danger, disconnect the device from the mains supply by pulling the power plug from the device or the mains socket or, by using the isolating device intended for this purpose (e.g., emergency stop switch in the laboratory).

WARNING! Damage due to UV radiation.
Microliter cuvettes, e.g., Hellma® TrayCell (or microliter cuvettes with a similar design) divert the radiation from the light source within the cuvette so the radiation can escape upward when the lid is not closed.

- Before starting a measurement, ensure that the lid on the microliter cuvette is not open.

WARNING! Damage to health from toxic, radioactive or aggressive chemicals as well as infectious liquids and pathogenic germs.

- Observe the national regulations for handling these substances, the biological security level of your laboratory, the material safety data sheets and the manufacturer's application notes.
- Wear personal protective equipment.
- For comprehensive regulations about handling germs or biological material of risk group II or higher, please refer to the "Laboratory Biosafety Manual" (source: World Health Organization, Laboratory Biosafety Manual, in its respectively current valid version).

WARNING! Damage to health due to contaminated device and accessories.

- Decontaminate the device and the accessories before storage and shipping.

CAUTION! Poor safety due to incorrect accessories and spare parts.
The use of accessories and spare parts other than those recommended by Eppendorf may impair the safety, functioning and precision of the device. Eppendorf cannot be held liable or accept any liability for damage resulting from the use of incorrect or non-recommended accessories and spare parts, or from the improper use of such equipment.

- Only use accessories and original spare parts recommended by Eppendorf.

2.3.2 Damage to device

NOTICE! Damage from the use of aggressive chemicals.

- Do not use any aggressive chemicals on the device or its accessories, such as strong and weak bases, strong acids, acetone, formaldehyde, halogenated hydrocarbons or phenol.
- If the device has been contaminated by aggressive chemicals, immediately clean it by means of a mild cleaning agent.

NOTICE! Damage to the device from fumigating with aggressive chemicals.

- Do not use fumigation to disinfect the device.

NOTICE! Corrosion from aggressive cleaning agents and disinfectants.

- Do not use corrosive cleaning agents, aggressive solvents or abrasive polishes.
- Do not incubate the accessories in aggressive cleaning agents or disinfectants for a longer period of time.

NOTICE! Damages and measurement errors due to condensate.
At high air humidity, condensate may form on a cuvette having a significantly lower temperature than the ambient temperature. The condensate may cause damage to the optics and lead to incorrect measuring results.

- Do not insert any cuvettes into the cuvette shaft with a temperature that is significantly lower than the ambient temperature.
- The temperature of the cuvette should not remain significantly below the ambient temperature for a longer period of time.
- If required, observe the actual dew point.

NOTICE! Damage to electronic components due to condensation.
Condensate can form in the device after it has been moved from a cool environment to a warmer environment.

- After installing the device, wait at least for 3 h. Only then connect the device to the mains.

NOTICE! Function impairment due to mechanical damage.

- After mechanical damage to the device, ensure that the measuring and evaluation functions of the device are operating correctly by completing an inspection.

NOTICE! Damage from overheating.

- Do not install the device near to any heat sources (e.g., heating, drying cabinet).
- Do not expose the device to direct sunlight.
- Ensure unobstructed air circulation. Keep free a clearance of at least 5 cm around all ventilation grilles.

NOTICE! Material damage from incorrect use.

- Only use the product for its intended purpose as described in the operating manual.
- Ensure adequate material resistance when using chemical substances.
- In case of doubt, contact the manufacturer of this product.

NOTICE! Damage as a result of incorrect packing.
Eppendorf AG is not liable for damage caused by improper packing.

- The device may only be stored and transported in its original packing.

NOTICE! Damage due to improper cleaning of the cuvette shaft.

- Only clean the cuvette shaft using a moist cotton swab (see *Cleaning on p. 77)*.
- Do not allow any liquid to enter the cuvette shaft.
- Do not reach with your fingers into the cuvette shaft.

2.4 Information on product liability

In the following cases, the designated protection of the device may be compromised. Liability for any resulting property damage or personal injury is then transferred to the operator:

- The device is not used in accordance with the operating manual.
- The device is used outside of its intended use.
- The device is used with accessories or consumables which are not recommended by Eppendorf.
- The device is maintained or repaired by people not authorized by Eppendorf.
- The user makes unauthorized changes to the device.

2.5 Safety instructions located on the device

Depiction	Meaning	Location
	Hazard point ▸ Follow the operating manual.	Rear side of the device
Gerät nach dem Öffnen justieren! Adjust device after opening!	The device needs to be readjusted after it has been opened. ▸ Do not open the device.	Bottom of the device

3 Product description

3.1 Main illustration

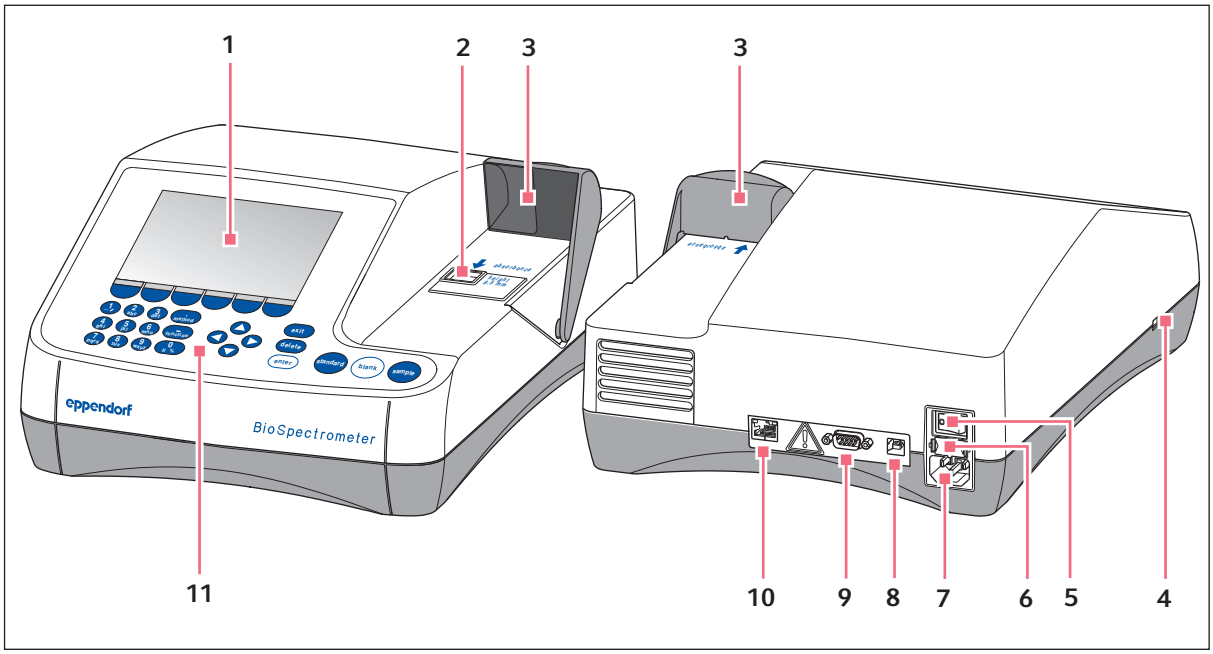


Fig. 3-1: Front and rear view

1 **Display**
2 **Cuvette shaft**
3 **Cuvette shaft cover**
4 **USB port for USB stick and printer**
5 **Mains/power switch**
6 **Fuse holder**
7 **Mains/power connection**
8 **USB port for PC**
9 **Connection for RS-232 printer**
10 **Ethernet connection socket**
11 **Operating controls**

The name plate is located at the rear left on the bottom of the device.

3.2 Delivery package

Quantity	Description
1	**BioSpectrometer kinetic**
1	**Power cord**
4	**4 UVettes** Original Eppendorf plastic cuvette, individually packaged, PCR clean, protein-free
1	**Operating manual, in multiple languages**

3.3 Features

The BioSpectrometer kinetic is a UV/Vis spectrophotometer for measuring liquids in cuvettes in a wavelength range of 200 nm to 830 nm. It is intended for use in development and research in the fields of molecular biology, biotechnology, biochemistry and cell biology. Glass and plastic cuvettes in a volume range of 1 µL to 3000 µL can be used.

3.3.1 Methods

Numerous methods for concentration determination of nucleic acids, proteins, and dye-marked nucleic acids and proteins, and the **OD 600** method for determining bacterial density via turbidity measurement, are already preprogrammed. Furthermore, method templates for various measurement and evaluation procedures (single and multiple wavelength measurements, acquisition of spectra, kinetic methods, evaluations with factor, standard and standard curve) are preprogrammed. It is possible to create individual methods on the basis of the preprogrammed methods and templates. The templates in the ***Absorbance*** method group can be used to quickly measure absorbances or spectra without an additional evaluation.

3.3.2 Operation

The preprogrammed methods and templates are combined into clearly arranged groups from which the desired method can be quickly selected. After calling up the method, you are guided through the measuring procedure in clear steps. A help box in the display provides hints upon request. The 3 round measuring keys (**standard**, **blank**, **sample**) allow users to quickly start a measurement.

3.3.3 Result output

The BioSpectrometer kinetic outputs the results via the device display or via a printer which is available from Eppendorf. With the USB connection, you can transfer result data from the device to a USB stick, a printer or directly to a PC. If the device is connected to a network, the results can be printed on a network printer or sent by e-mail. The results cannot be saved to a network drive.

3.3.4 Device self test

The device automatically checks the function of the spectrometer unit and thermal module immediately after it has been switched on. Access the **Device calibration** function for a more comprehensive test (see *Device self test on p. 84)*.

4 Installation

4.1 Preparing installation

- Keep the transport carton and the packing material for subsequent safe transport or storage.
- Check the completeness of the delivery using the information in the delivery package (see *Delivery package on p. 15)*.
- Check all parts for any transport damage.

4.2 Selecting the location

Select the location for the BioSpectrometer kinetic according to the following criteria:

- 2 grounded sockets for the BioSpectrometer kinetic and for the printer.
- Solid laboratory bench with horizontal work surface
  Space requirement of the device: 50 cm (with printer: 75 cm) width, 50 cm depth.
- Temperature: 15°C to 35°C.
- Avoid temperature fluctuations (e.g, caused by open windows).
- Avoid direct sunlight.
- Humidity: 25% to 70% relative humidity.

Ensure that no objects (e.g., loose sheets, notebooks) that could impede the flow of air are positioned under the device.

4.3 Connecting the device to the mains

1. Place the BioSpectrometer kinetic on a suitable work surface.
2. Verify that the mains/power supply voltage and mains/power frequency match the information on the name plate.
3. Connect the device to the mains/power line and switch it on with the power switch.
4. Remove the protective film from the display.

4.4 Connecting the device to a network

Connecting the device to a network is optional. You can also operate the device without a network connection.
Information on network settings (see *Device settings on p. 72)*

Prerequisites

Ethernet cable (RJ45)

1. Connect the Ethernet cable to the network connection socket.
2. Connect the Ethernet cable to the Ethernet connection socket **10** (see *Main illustration on p. 15)*.

Network printer
A network printer is recognized automatically by the device if the following prerequisites have been met:
- The printer is in the same network segment as the device.
- The printer supports the Zeroconf protocol.
- The printer has PostScript enabled.

4.5 Connecting the printer to the USB port

4.5.1 Thermal printer DPU-S445

Prerequisites

Software version 3.4.4.0 or higher is installed on the device.

The thermal printer DPU-S445 has been selected in the printer settings (see *Device settings on p. 72)*.

Connect the thermal printer DPU-S445 to the USB port for printers.

1. Connect the printer cable with the USB port for printers **4** (see *Main illustration on p. 15)*.
2. Connect the printer cable with the printer.
3. Connect the printer to the mains/power line using the supplied mains/power adaptor and mains/power cord (printer accessory) and switch it on.

   For information on the printer, refer to the operating manual of the printer.

4.6 Connecting PC or USB stick for data export

You can connect a **FAT 32-formatted** USB stick to the USB port **4** (see *Main illustration on p. 15)*.

Alternatively, you can connect the device for the data export directly to a PC by using a USB cable:

Prerequisites

- PC with Windows, version XP, SP2 or higher version.
- USB cable with a type A and type B plug each.

▸ Connect the device to the PC by using the USB cable on the USB port **8** (see *Main illustration on p. 15)*.

- You do not need any special PC software for the data transmission: the transferred data packets are recognized by the PC like a USB stick as a removable medium. For viewing the data, you only need to open the registered data packet.
- The transmission of data to the USB stick or to the PC is started after completing the series of measurement in the **print & export** (see *Print & export on p. 62)* method step.

5 Operation

5.1 Overview of operating controls

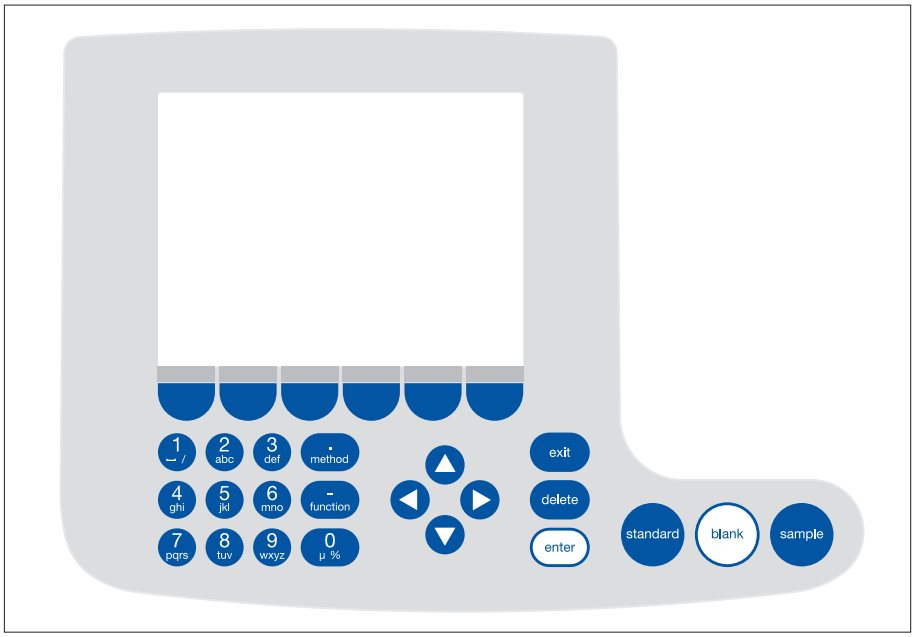
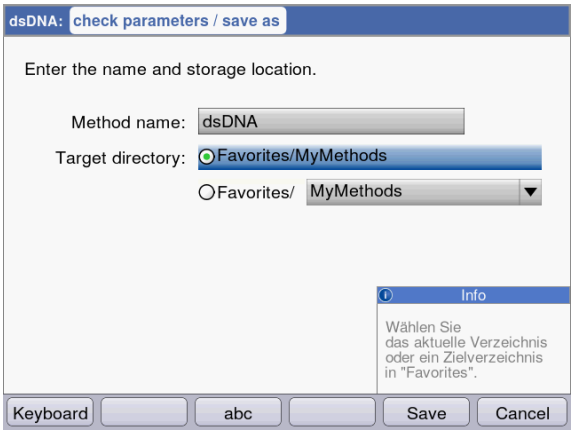


Fig. 5-1: Control panel of the BioSpectrometer kinetic

Key:	Function
1 ␣ / 2 abc 3 def · method 4 ghi 5 jkl 6 mno - function 7 pqrs 8 tuv 9 wxyz 0 µ %	Keypad: Enter digits and text. Keys **1** to **9** as well as **0**: When entering text, next to numbers you also can enter letters and special characters by pressing the key several times. Alternatively, you can switch to a displayed keyboard with the [Keyboard] key.
· method - function	Outside of entry fields: Call up method selection. Outside of entry fields: Call up function selection.
Edit	Softkey: Select functions. The key assignment changes along with the software dialog. The current function is displayed directly above the key on the display.
◀ ▲ ▶ ▼	Move the cursor to the left, right, up, down. • Navigation between input fields. • ◀ and ▶ keys inside an entry field: Navigate within the character string. • ▲ and ▼ keys in a result display: Navigate between the sample results of the series of measurement. • ◀ and ▶ keys within a graph: Navigate on the x-axis of the graph, e.g. for displaying the wavelength-dependent absorbance values in a scan. ▲ and ▼ keys in an absorbance wavelength spectrum: Change image section (**SpectraZoom** procedure) (see Tab. on p. 58).
exit delete enter	Exit the current selection for the next higher level. Delete entry. Within a sequence of signs, the sign on the left of the cursor is deleted • Call up selected method or function. • Open the selection list. • Confirm entry or selection.
standard blank sample	Start standard measurement. Start blank measurement. Start sample measurement.

5.1.1 Entering text

You can enter texts when assigning method names and result units. Restriction: Only digits, letters and the underscore "_" are allowed for method names.



Entry via keyboard:
Use the ◀ and ▶ cursor keys to navigate within the entry field and to change single positions in the name.
Softkeys:

- [Keyboard]: Display keyboard.
- [abc]: Change between upper and lower case letters when making entries with the keypad.
- [Save]: Save entered text.
- [Cancel]: Cancel text input.

Entry via the displayed keyboard:
Use the cursor keys to select the displayed signs and respectively confirm your selection with the **enter** key. As for a PC key pad, you can use the "Shift" resp. the "Caps Lock" key for changing the capitalization for the next entry or for all following entries.
Softkeys:

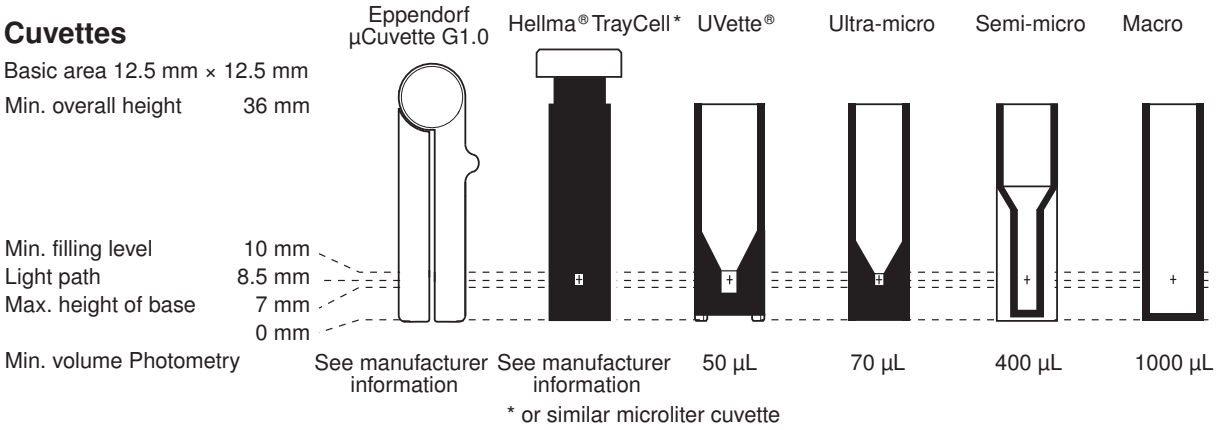
- [Numbers]: Switch to entry using the keyboard.
- [Save]: Save entered text.
- [Cancel]: Cancel text input.

5.2 Inserting the cuvette

Standard rectangular glass or plastic cuvettes can be inserted in the cuvette shaft:

- External dimensions: 12.5 mm × 12.5 mm
- Height of light path: 8.5 mm higher than cuvette base
- Total height: min. 36 mm

The cuvettes must be optically transparent for the respective measuring wavelength. For measurements in the UV range, Eppendorf offers the plastic cuvette UVette which is transparent for wavelengths of 220 nm and higher and therefore also is suitable for measuring nucleic acids.



Prerequisites

- The cuvette is free from contamination by dust or fingerprints and free from scratches.
- The cuvette shaft is free from particles, dust and liquid.
- The measuring volume in the cuvette is sufficient. Ensure that the minimum measuring volume has been reached.
- The measuring solution is free from particles and bubbles.

The direction of the light path is marked with an arrow on the housing.

1. Position the cuvette so that the optical window of the cuvette is pointing towards the direction of the light path.
2. When inserting the cuvette, press it completely to the bottom against the slight resistance.

5.3 Summary of the measuring procedure

5.3.1 Preparing the measurement

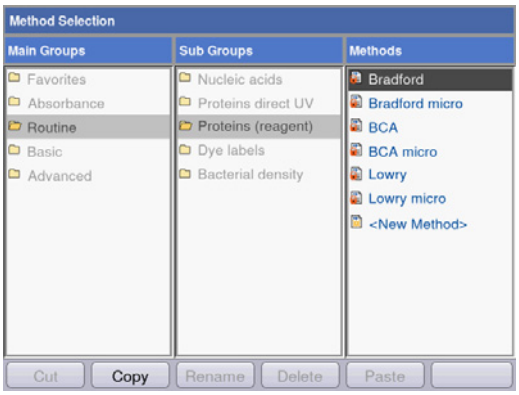
1. Switch on the device and, if required, the printer.

   The device performs a self test (taking approx. 1 minute) and displays the method selection.
2. Make ready the cuvettes for the measurements (see *Inserting the cuvette on p. 24)*.
3. Prepare the measuring solutions for measuring the blank values, if required, also the standards and the samples.
4. Open the cover of the cuvette shaft. The cover can remain open during the measurements.

Measuring solutions for standards and samples with absorbance values lower than 0.05 A should not be used. The detection limit of the device may be significantly lower, nevertheless, the impact of disturbances from the measuring solutions (e.g. particles, bubbles, turbidity) on the reliability of the result is very high for these low absorbance values. Further information such as the Userguide no. 013 can be found on our webpage www.eppendorf.com.

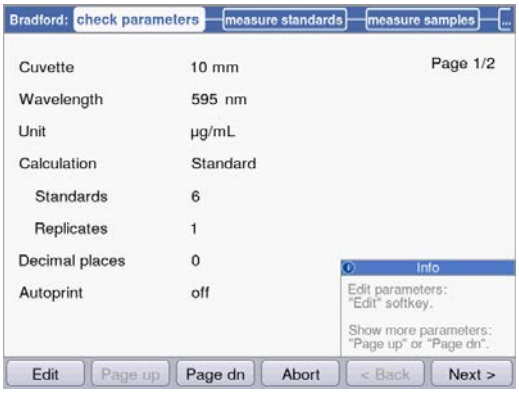
5.3.2 Measuring procedure

5.3.2.1 Selecting a method



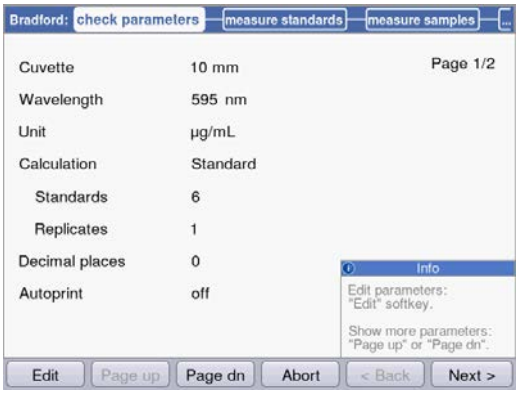
- Use the cursor keys to select the desired method and call up the method with the **enter** key.

For an overview and a detailed description of the methods, refer to the next chapter (see *Methods on p. 33)*.



Wizard: The wizard at the top of the display will take you through the method procedure step-by-step.
Help box: You will receive help texts in the lower right of the display during each step of the procedure.
Softkeys: The [< Back] and [Next >] softkeys allow you to move between method steps in the wizard.

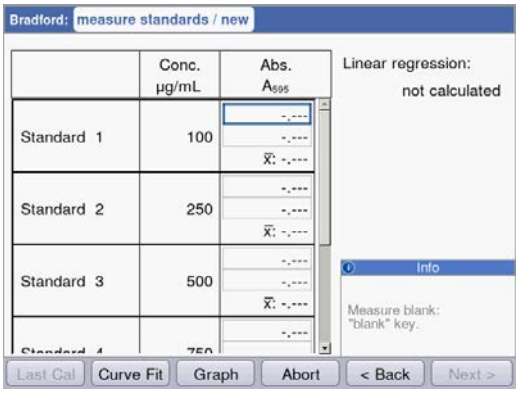
5.3.2.2 Checking parameters



- Check the parameter setting. The [Page dn] and [Page up] softkeys allow you to call up the parameter list pages. You can modify and save parameters using [Edit].

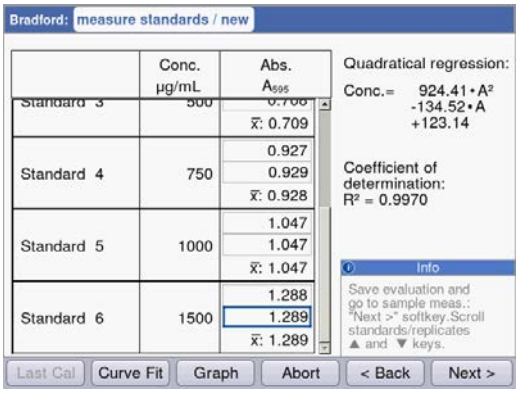
5.3.2.3 Measuring the blank and standards

For evaluations without standards (e.g., DNA measurements), this method step is omitted.



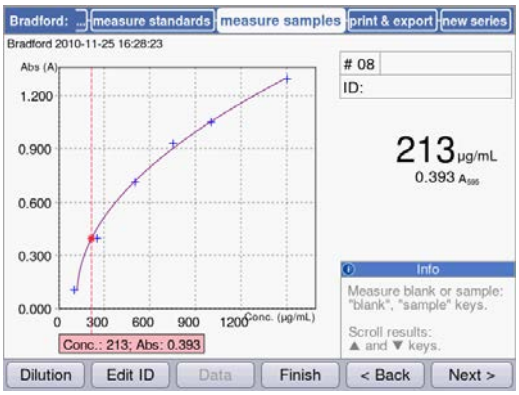
1. Start by measuring a blank (**blank** key).
2. Then measure all standards one by one (**standard** key).

The display always marks the standard that is to be measured next. Use the [Graph] resp. [Table] softkey to change the result view.



- Press [Next] to accept the evaluation calculated from the standard results.

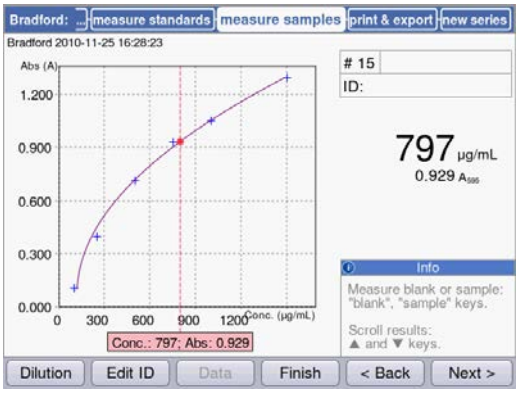
5.3.2.4 Measuring samples



- The **sample** key is used for measuring your samples consecutively.

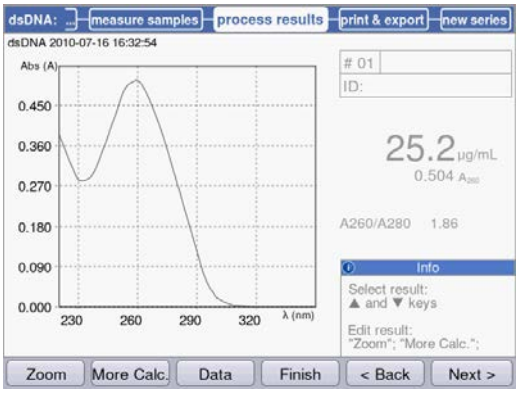
Blank results will remain saved for the duration of one series of measurement. However, a new blank measurement always is possible. (The adjacent figure shows a measuring procedure with evaluation via the standard curve and, in addition to the sample result, the graph of the standard evaluation.)

5.3.2.5 Finalizing the method



1. Press [Finish], to complete the measuring series and return to the method selection.
2. After all measurements have been completed, switch off the device and close the cuvette shaft cover to protect the cuvette shaft from contamination.

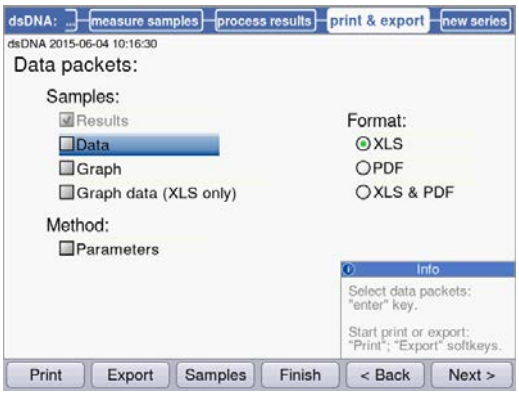
5.3.2.6 Optional: process results



For some methods, you can postprocess the results in the **process results** method step. For example, you can use the **SpectraZoom** zoom function in the spectra.

- Use the ▲ and ▼ cursor keys for selecting systematically any results of the series of measurement for postprocessing.

5.3.2.7 Printing and exporting



1. Compose data packets for all samples or for selected samples.
2. Print the data, save them to a USB stick, transfer them to a PC via a USB cable or export them via e-mail.

5.3.3 Important measurement instructions

Check for each measurement:

- For plastic cuvettes: How many consecutive measurements can be reliably carried out in the cuvette?
- Measure the cuvette blank value before the sample or standard measurements in order to compensate the cuvette blank in addition to the reagent blank value.
- Blank results remain saved for one measuring series, but a new blank result measurement can be performed at any time, even between sample measurements.
- The displayed absorbance values always correspond to the directly measured values. The dilution or cuvette factor as well as background absorbances only will be incorporated for the following result calculation (see *Absorbance values on p. 103)*.
- The measuring result is typically displayed 2 to 3 seconds after a measurement has been started. If a small amount of light reaches the receiver, the measuring time automatically can be extended to 9 seconds in order to increase the precision of the measurement. For kinetic measurements, the automatic extension of the measuring time is not applied in order to prevent any conflicts with the preprogrammed interval time for the measuring point recording.
- Observe that the measured absorbance values do not exceed the upper limit of the photometric measuring range. In this case, reject the measuring result. The upper limit of the photometric measuring range does not only depend on the wavelength (see *Photometric properties on p. 100)* but also on the cuvette blank. Ultra-micro cuvettes with a small diaphragm, such as **TrayCell** (Hellma), may have a cuvette blank of approx. A = 1. The available photometric measuring range is reduced by this amount. You can estimate the cuvette blank by measuring the cuvette filled with demineralized water as a sample in comparison with the empty cuvette shaft as a blank. The cuvette blank of the Eppendorf µCuvette G1.0 is negligible (approximately A = 0).
- After the measurement, remove the measuring solution completely before filling in the next measuring solution in order to minimize carry-over. If a carry-over from one sample to the next sample can be expected due to a high concentration difference, rinse the cuvette between the measurements.
- If the temperature between the lamp and the ambience differs, photometric drift may occur. Therefore a device from a colder ambience first has to be adjusted to the ambient temperature.
  Avoid quick changes of temperature. Carry out a new blank measurement for a long series of measurements or measurements over a long period of time.

5.3.4 Notes on working with cuvette temperature control

Temperature control is regulated using a measurement on the cuvette holder. The temperature in the measuring solution may deviate from the temperature on the cuvette holder.

The extent of the deviation is dependent on the measuring volume, cuvette material, cuvette shape and ambient temperature. The temperature control speed is also dependent on these factors. The temperature control of plastic cuvettes is slower compared to glass cuvettes. The surface of the cuvette in direct contact with the wall of the cuvette holder should be as large as possible to ensure quick temperature control. Therefore, the temperature control of plastic semi-micro cuvettes as well as, for example, the UVette is only performed slowly.

Measured values typical for Eppendorf for temperature control in closed cuvettes, with closed cuvette shaft covers, are shown in the following tables. The temperature was measured in the measuring solution; the ambient temperature was 24.5°C.

Tab. 5-1: The temperature to be set for temperature control of a measuring solution

Cuvette type/measuring volume	**Target temperature**	**Temperature to be set in the method parameters**
Quartz glass macrocuvette, 1 500 µL	25°C	25°C
	30°C	30.4°C
	37°C	37.7°C
Plastic macrocuvette, 1 000 µL	25°C	25°C
	30°C	30.4°C
	37°C	37.7°C
Quartz glass semimicrocuvette, 500 µL	25°C	25°C
	30°C	30.4°C
	37°C	37.7°C
Quartz glass ultra-micro cuvette, 60 µL	25°C	25°C
	30°C	30.3°C
	37°C	37.6°C

Tab. 5-2: Duration for temperature control of a measuring solution

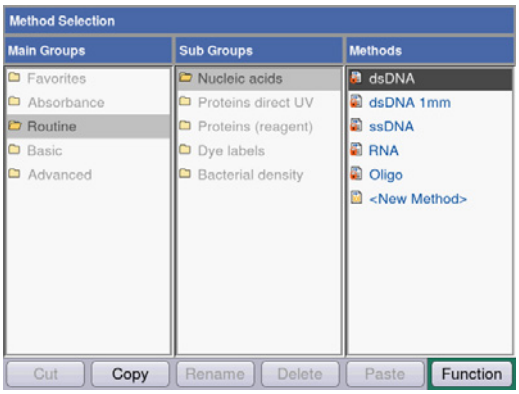
Cuvette type/measuring volume	Initial temperature and target temperature	Temperature control duration
Quartz glass macrocuvette, 1 500 µL	25°C → 37°C	Approx. 7 min
	37°C → 25°C	Approx. 11 min
	25°C → 30°C	Approx. 7 min
Plastic macrocuvette, 1 000 µL	25°C → 37°C	Approx. 13 min
	37°C → 25°C	Approx. 19 min
Quartz glass semimicrocuvette, 500 µL	25°C → 37°C	Approx. 7 min
	37°C → 25°C	Approx. 12 min
Quartz glass ultra-micro cuvette, 60 µL	25°C → 37°C	Approx. 7 min
	37°C → 25°C	Approx. 9 min
	25°C → 30°C	Approx. 5 min

- For efficient temperature control, the volume of the measuring solution in the cuvette should not project beyond the edge of the cuvette holder.
- To speed up the measuring procedure during series measurements, you can pre-cool cuvettes with reagents in a thermostat outside the BioSpectrometer before inserting the cuvette in the cuvette holder and adding the sample.
- When switching from a method with temperature control to one without temperature control, please note that the temperature of the cuvette holder will slowly drift back to room temperature. The results of the method without temperature control could be affected by this.

6 Methods

6.1 Selecting a method

Methods and method templates are delivered preprogrammed. The methods are organized in main groups and subgroups.



Write-protected methods		The most important methods in molecular biology. Parameters can be modified, but the modified parameters must be saved under a new method name.
Non-write-protected methods		You can change parameters any number of times and start the measurement right after saving.
New methods ("templates")		Each method group contains a template which is preprogrammed with complete parameter sets to facilitate the programming of new methods. The parameters can be changed and saved under new names any number of times.

To call a method, first use the cursor keys to select the main group, subgroup and the method. Confirm each with **enter**.

Tab. 6-1: Photometric methods

Absorbance	Methods for fast, simple absorbance measurements without additional evaluations
Routine	Frequently used molecular biology methods. The methods are preprogrammed. However, the parameters can be modified if saved under a new name.
Basic	Methods for the evaluation of absorbance measurements with factor, standard or standard curve/line. Method templates for measuring and evaluating kinetics.
Advanced	Methods for the evaluation of two wavelength measuring methods and for kinetics with more demanding evaluation options.
Favorites	In ***Favorites***, you can set up your own folders using **<New folder>**, and copy your frequently used methods to this folder in order to quickly access them when needed.

You can create new methods in all folders using **<New Method>**.

In ***Favorites***, you can create your own folders (e.g., to allocate folders to specific people), and rename and delete the folders.

Tab. 6-2: Softkeys in method selection

[Cut] and [Paste]	Cut and paste methods.
[Copy] and [Paste]	Copy and paste methods.
[Delete]	Delete methods.
[Rename]	Rename methods.

Copied or cut methods can be added to a different folder under ***Favorites***, or added to the original folder under a new name. Use the cursor keys to navigate to the **Methods** column of the desired folder and press [paste] for adding the method.

6.2 Photometry method description

The preprogrammed methods and method templates are described in this section.

6.2.1 *Absorbance* method group

Single λ
- Absorbance measurement on a wavelength.
- No subsequent evaluation.

Single λ - continuous
- Repeated absorbance measurement on a wavelength.
- Parameters can be entered for temperature control between 20 and 42°C (presetting: 37°C).
- Entry of parameters for total time and interval time for the measuring points. A premature stop cannot be made during the measurement.
- Evaluation as kinetic via **linear regression**. A subsequent change cannot be made to the time frame for the evaluation.
- The measured values are displayed in an absorbance time graph.
- To subsequently evaluate measured values as linear regression kinetics, press the [Next >] softkey and go to the **process results** method step.

Multi λ
- Absorbance measurement at two to six wavelengths.
- No subsequent evaluation.

Scan
- Absorbance wavelength spectra measurement via a defined wavelength range.
- Display of wavelength and absorbance in the spectrum by navigation with a wavelength cursor.
- The spectra section can be modified using 3 different zoom variants.
- Peak detection possible.

6.2.2 *Routine* method group

The methods for the ***Routine*** group are preprogrammed as fixed methods. Therefore, a new method name is required after the method parameters in the fixed preprogrammed methods have been modified.

Nucleic acids

- Determination of the concentration of nucleic acids through measurement at 260 nm and evaluation via factor.
- Various nucleic acid methods, such as dsDNA or RNA, are preprogrammed. The parameters vary according to the factor.
- Preprogrammed method for microliter cuvettes: Measuring DNA in sample volumes within the microliter range with 1 mm light path (with microliter cuvettes as Eppendorf µCuvette G1.0 or Hellma® TrayCell).
- Additional information on the purity of the measured nucleic acid: Ratios A260/A280, ratios A260/A230, absorbance wavelength spectrum of nucleic acid, absorbance of the background wavelength (preset: 320 nm; the absorbance of the pure nucleic acid should be close to zero here).
- Partial turbidity correction can be performed via the **Background** parameter.
- Concentrations can be converted to molar concentrations and (after the sample volume has been entered) to nucleic acid quantities (**process results** method step).

Proteins direct UV

- Determination of the concentration of proteins via measurement at 280 nm and factor or standard evaluation.
- Preprogrammed methods for direct absorbance output as a result *(Protein A 280)* and for evaluation via albumin-specific absorbance coefficients *(Albumin A 280).*
- Preprogrammed method for microliter cuvettes: Measuring protein in sample volumes in the microliter range with 1 mm light path (with microliter cuvettes as Eppendorf µCuvette G1.0 or Hellma® TrayCell).
- Additional information on the purity of the measured protein: Absorbance of the background wavelength (preset: 320 nm; the absorbance of the pure protein should be close to zero here).
- Partial turbidity correction can be performed via the **Background** parameter.
- When programming the methods, the corresponding factor is imported through the simple selection of the protein from a predefined list. The factors are separately defined in the functions of the **Gen. method param.** group. Various proteins are preprogrammed in **Gen. method param.**; additional proteins can be added.

Proteins (with reagent)

- Concentration determination of proteins via measurement according to color reactions and evaluation using standards or factors (typical: evaluation with standard curve).
- The *Bradford*, *Bradford micro*, *Lowry*, *Lowry micro*, *BCA and BCA micro* methods are already preprogrammed. According to the reagent manufacturer, the "Curve fit" (standard curve type) must be changed as necessary.

Dye labels

- For dye-labeled biomolecules: Concentration determination of the biomolecule (nucleic acid or protein) via measurement at 260 or 280 nm and measurement of the dye in one measuring procedure.
- Evaluation with factor. In addition to the biomolecule, up to two dyes can be measured at the same time as two different wavelengths.
- Additional: evaluation of the frequency of incorporation (FOI) of the dye. Selection between two different FOI calculation procedures.
- Already preprogrammed methods: *ssDNA,* labeled with *Cy 3* or *Cy 5*.
- Correction of the influence of the dye spectrum on the accuracy of the biomolecule measurement is possible.
- Partial turbidity correction can be performed via the **Background** parameter.
- Additional information on the purity of the measured materials: Ratios A260/A280 and ratios A260/A230 (ratio values only for nucleic acids), absorbance wavelength spectrum.
- When the methods are programmed, various associated parameters, such as wavelengths and evaluation factors, are imported by simply selecting the biomolecule and the dye from predetermined lists. These parameters are separately defined in the functions of the **Gen. method param.** group. Various nucleic acids, proteins and dyes are preprogrammed in **Gen. method param.**. You may add further nucleic acids, proteins and dyes.
- Only for labeled nucleic acids: Concentrations can be converted to molar concentrations and (after the sample volume has been entered) to nucleic acid and dye quantities (**process results** method step).

Bacterial density

- Turbidity measurement to determine the bacteria density.
- Measurement at 600 nm is already preprogrammed.
- Additional information: absorbance wavelength spectrum.

6.2.3 *Basic* method group

Factor, standard

- Measurement on a wavelength and factor or standard evaluation.
- Methods for factor and standard evaluation are preprogrammed.

Calibration curve

- Measurement on a wavelength and subsequent evaluation with a series of 2 to 12 standards.
- You can select between different evaluation procedures ("Curve fit") as linear regression, non-linear regression.
- Graphical and tabular display of the standard results.
- The last saved standard evaluation can be used.
- A method for standard curve evaluation is preprogrammed.

Simple kinetics

- Measurement of a kinetics at one wavelength and subsequent evaluation by factor.
- 3 different measurement methods are available:
  - "Linear regression": Evaluation in a series of measuring points recorded in intervals.
  - "Two point": Calculation of ΔA/min using 2 measuring points at defined times.
  - "Endpoint": Recording of a measuring point at a defined time.
- Parameters can be entered for temperature control between 20 and 42°C (presetting: 37°C).
- For the "linear regression" measurement method, the absorbance/time curve is depicted as a graph.
- A method for the evaluation with linear regression is preprogrammed and can be changed for other evaluation procedures ("endpoint", "two.point") via simple selection.

6.2.4 *Advanced* method group

Dual wavelength

- Measurement at two wavelengths and evaluation of the measured absorbance values via two basic formulas (subtraction, division)
- Variants of the basic formulas can be defined.
- The result can be evaluated with a factor, with a standard or with a standard series.
- Methods are preprogrammed for calculation, subtraction and division, and subsequent factor evaluation.

Advanced kinetics

The following options are available via the description of the *Simple kinetics* method (***Basic*** method group):

- Measurement of a reagent blanks kinetics. The blank result is subtracted from all sample results prior to the evaluation.
- An alternative to evaluation via factor is evaluation via a standard.

6.3 Method parameters

This chapter illustrates the parameters for programming the methods. The order of the parameters in the device display may slightly differ from the order in the table in order to display the parameters more clearly. The table displays all parameters available for the various methods. Only a small portion of these parameters are required for the corresponding method and will be shown in the display.

Parameter	Entry	Explanation
Cuvette	Selection: 10 | 5 | 2 | 1 | 0.5 | 0.2 | 0.1 mm	Optical path length of the cuvette. The device always automatically converts absorbance values to the 10 mm path length of a standard cuvette (see *Absorbance values on p. 103)*. Therefore, there is no need to change factors such as "50" for the calculation of dsDNA concentrations when modifying the **Cuvette** parameter.
No. of wavelengths	Value input: Range: 2 to 6.	Only for the **Multi λ** method group. Number of wavelengths at which the measurement is to be performed.
Wavelength	Value input: Measurement wavelength in nm. Range: 200 to 830 nm.	Measurement wavelength: The concentration is calculated based on the absorbance measured with this wavelength. For the **Multi λ** and **Dual wavelength** method groups, enter more than one wavelength. For some method groups (e.g., **Nucleic acids** and **Proteins direct UV**), the wavelengths are preprogrammed. For the **Dye labels** method group the measuring wavelengths are not entered into the method procedure one by one. They are automatically imported simply by selecting the biomolecule and dye from the **General Method Parameters** function.
Unit	Selection: mg/mL | µg/mL | ng/mL | pg/mL | µg/µL | mg/dL | µmol/mL | nmol/mL | pmol/mL | pmol/µL | U | U/mL | U/L | % | Abs | A/min In addition, further units are freely programmable in the **General Method Parameters/Units** function. Max. 7 digits.	Unit for the concentration result. In the preprogrammed methods of the ***Routine*** group, the selection is restricted to units that are useful for these methods.
Formula type	Selection: division | subtraction	Only for the **Dual wavelength** method group. Formula type for calculating the absorbances at the two measurement wavelengths prior to evaluation with factor or standard.

Parameter	Entry	Explanation
Formula: *a*	Value input: Value for *a* in the evaluation formula. Limit: max. of 5 digits including decimal point.	Only for the **Dual wavelength** method group. Value for *a* in the formulas: *[(a*A1) / (b*A2)] * c + d* and *[(a*A1) - (b*A2)] * c + d*.
Formula: *b*	Value input: Value for *b* in the evaluation formula. Limit: max. of 5 digits including decimal point.	Only for the **Dual wavelength** method group. Value for *b* in the formulas: *[(a*A1) / (b*A2)] * c + d* and *[(a*A1) - (b*A2)] * c + d*.
Formula: *c*	Value input: Value for *c* in the evaluation formula. Limit: max. of 5 digits including decimal point.	Only for the **Dual wavelength** method group. Value for *c* in the formulas: *[(a*A1) / (b*A2)] * c + d* and *[(a*A1) - (b*A2)] * c + d*.
Formula: *d*	Value input: Value for *d* in the evaluation formula. Limit: max. of 5 digits including decimal point.	Only for the **Dual wavelength** method group. Value for *d* in the formulas: *[(a*A1) / (b*A2)] * c + d* and *[(a*A1) - (b*A2)] * c + d*.
Calculation	Selection: factor | standard	Evaluation procedure for the calculation of the sample concentration from the measured absorbance.
Factor	Value input: Factor. Limit: max. of 6 digits including decimal point.	Factor for converting absorbance values into the concentration. Negative factors can also be entered for the following method groups: **Simple kinetics, Advanced kinetics, Dual wavelength, Factor**. For the **Dye labels** method group the factors are not entered into the method procedure individually. They are automatically imported simply by selecting the biomolecule and dye from the **General Method Parameters** function.
Protein	Selection: List of protein types which are stored in the **General Method Parameters/Proteins** function.	Only for the **Dye labels** and **Proteins direct UV** method groups. When selecting the protein, the corresponding **Factor** parameter programmed in the **General Method Parameters/ Proteins** function also will be imported from that function.
Standards	Value input: Number of standards. Range: 1 to 12.	Number of different standard concentrations for the evaluation with standards. For some methods the range for the number of standards is restricted to a smaller range than 1 to 12.

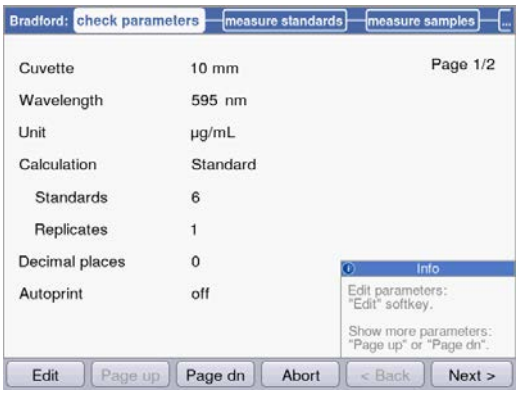
Parameter	Entry	Explanation
Replicates	Value input: Number of replicates per standard. Range: 1 to 3.	Number of repeated measurements for the various standard concentrations.
Std. conc.	Value input: Concentration values of the standards. Limit: max. of 6 digits including decimal point.	Based on the number of standards, this parameter is available for all standards (e.g.,: std. conc. 1, std. conc. 2, ...).
Decimal places	Value input: Number of decimal points for the result. Range: 0 to 3.	Number of decimal points for the calculated concentration result.
Dye 1	Selection: List of dyes that are stored in the **General Method Parameters/ Dyes** function.	Only for the **Dye labels** method group. When the dye is selected, the parameters programmed in the **General Method Parameters/Dyes** function, which correspond to the dye, are also imported: factor, wavelength and, if necessary, correction factors for the measurement at 260 or 280 nm (see the description of the following parameter).
Correct A260 1	Selection: on | off	Only for the **Dye labels** method group. Correction of the influence of the dye spectrum on the absorbance with the measuring wavelength of the biomolecule (260 nm or 280 nm). Some of the dye spectra have a low absorbance at 260 and 280 nm. These absorbances distort the calculations for the nucleic acids or the proteins of these methods. To minimize the distortion correction factors are used, as long as these are known for the corresponding dyes. When the parameter is switched on, the correction factor is imported from the **General Method Parameters/Dyes** function.
Correct A 280 1	Selection: on | off	Only for the **Dye labels** method group. For an explanation see the description of the **Correct A260 1** parameter above.
Dye 2 active	Selection: on | off	Only for the **Dye labels** method group. Option to measure a second dye at the same time. Application: Labeling a biomolecule with two dyes.
Dye 2	Selection: List of dyes that are stored in the **General Method Parameters/ Dyes** function.	Only for the **Dye labels** method group when measuring 2 dyes. Selection of the second dye (see **Dye 1** parameter).
Correct A260 2	Selection: on | off	Only for the **Dye labels** method group when measuring 2 dyes. Analog to parameter **Correct A260 1**.
Correct A280 2	Selection: on | off	Only for the **Dye labels** method group when measuring 2 dyes. Analog to parameter **Correct A280 1**.

Parameter	Entry	Explanation
Show scan	Selection: on | off	Display of a scan (absorbance wavelength graph) in addition to the result of the sample measurement.
Start λ	Value input: Wavelength in nm. Range: 200 to 830 nm.	Starting wavelength for recording the scan.
Stop λ	Value input: Wavelength in nm. Range: 200 to 830 nm. Value must be higher than the value for **Start λ**.	Stopping wavelength for recording the scan.
A260/A280	Selection: on | off	Only for nucleic acids. Display of the A260/A280 ratio in addition to the result of the sample measurement.
A260/A230	Selection: on | off	Only for nucleic acids. Display of the A260/A230 ratio in addition to the result of the sample measurement.
FOI	Selection: none | dye/kb | pmole/µg	Only for the **Dye labels** method group. Display of the FOI in addition to the result of the sample measurement. The FOI (frequency of incorporation) is a measure for the number of dye molecules per nucleic acid molecule that are integrated into the nucleic acid. Units are "dye/kb" (dye molecules per 1000 bases) or "pmole/µg" (pmol dye per µg nucleic acid). "None": no FOI calculation.
Background	Selection: on | off	Prior to the calculation of the results of a sample the absorbance of a background wavelength, during which the analyte to be measured should exhibit the absorbance value zero, is subtracted from the absorbance of the measuring wavelength. Frequent application: Partial correction of turbidity for measurement of nucleic acids (background wavelength in this case: 320 nm or 340 nm).
Wavelength	Wavelength in nm. Range: 200 to 830 nm.	Wavelength at which the background is to be measured. The analyte to be measured should have the absorbance value zero in pure form here.
Background for dyes	Selection: on | off	Only for the **Dye labels** method group. Application of the background correction to the measurement of a dye (see **Background** parameter).
Wavelength	Wavelength in nm. Range: 200 to 830 nm.	Only for the **Dye labels** method group. Wavelength at which the background for the dye is to be measured. The pure, not contaminated dye to be measured should have the absorbance value zero for this wavelength.
Temperature on	Selection: on | off	For kinetic methods only. Use of cuvette temperature control.

Parameter	Entry	Explanation
Temperature	Value input: Temperature in °C. Range: 20 to 42°C.	For kinetic methods only. Temperature input for the cuvette temperature control when the **Temperature** parameter is set to "on". **Notice! Damages and measurement errors due to condensate.** At high air humidity, condensate may form on a cuvette having a significantly lower temperature than the ambient temperature. The condensate may cause damage to the optics and lead to incorrect measuring results. ▸ The temperature of the cuvette should not remain significantly below the ambient temperature for a longer period of time. If required, observe the actual dew point.
Measuring procedure	Selection: lin.regr. | endpoint | two point	For kinetic methods only. "Linear regression": Measurement over several periods in set time intervals within a defined period of time. Evaluation via linear regression of the absorbance time graph within the measuring time. Absorbance result: ΔA/min. "Endpoint": Measurement of a measuring point after a defined period of time. Absorbance result: A. "Two point": Measurement of 2 measuring points at defined times. Evaluation: Via linear interpolation between these measuring points in the absorbance time graph. Absorbance result: ΔA/min.
Reagent blank	Selection: on | off	For kinetic methods of the **Advanced kinetics** method group and evaluation with factor only. Measurement of a reagent blank (RB). The RB is measured according to the same measurement method as a sample. The absorbance result in A or ΔA/min is subtracted from the absorbance result of the sample before the sample concentration is calculated. Application: Correction of sample results for kinetics with reagent drift. The reagent blank contains the reagent and demineralized water as sample.
Delay	Value input: Time from the start to the first measuring point. Range: 00:00 to 10:00 min:sec.	For kinetic methods only. Time from the start of the measuring procedure to recording the same measuring point.
Measuring time	Value input: Time between first and last measuring point. Range: 00:05 to 59:59 min:sec.	Only for kinetic methods and the "lin.regr." and "two point" measurement methods. Time between recording the first and the last measuring point.

Parameter	Entry	Explanation
Total time	Value input: Time between first and last measuring point. Range: 00:05 to 59:59 min:sec.	For the **Single λ - cont** kinetic method only. Time between recording the first and the last measuring point.
Interval	Value input: Time between two measuring points. Range: 00:05 to 10:00 min:sec.	Only for kinetic methods and "lin.regr." measurement methods. Time intervals between the measuring points.
Autoprint	Selection: on | off	Printing a measuring result immediately following measurement with the thermal printer. Only the main result data will be printed. To output detailed data, the required data packets can be compiled and printed in the **print & export** method step at the end of a measuring series.

6.4 Method procedure



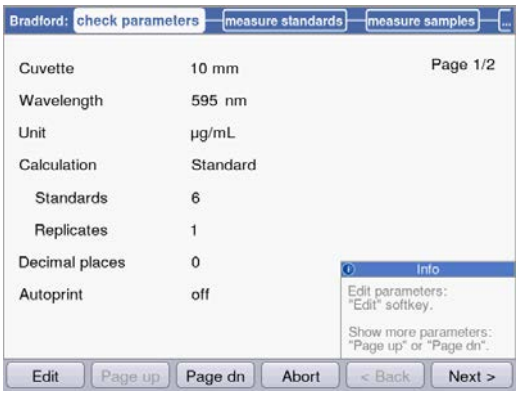
Wizard: the wizard at the top of the display will take you through the method procedure. The currently active method step is highlighted.

A method procedure is composed of a maximum of 5 steps. The currently active step is highlighted visually. After the last step, **print & export**, of a measuring series, the start of a new measuring series is offered as a next step. It once again starts with the sample measurement.

Method step	Explanation
Check parameters	Check method parameters. Carry out changes if required.
Measure standards	Only for methods with standard evaluation: Measure and evaluate standards. Alternatively, the last standard evaluation saved can be used.
Measure samples	Measuring samples
Process results	Only for some methods: postprocess results, e.g. zoom scan graphs.
Print & export	Assemble data packets for printing or exporting the data.

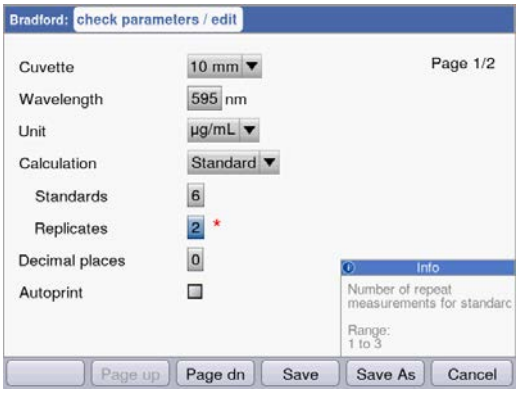
Use the [Next >] and [< Back] softkeys to navigate between method steps. With [Abort] and [Finish] you can cancel or finish the measuring procedure. The name of this softkey changes from [Abort] to [Finish] after the first sample measurement.

6.4.1 Check parameters



Softkeys

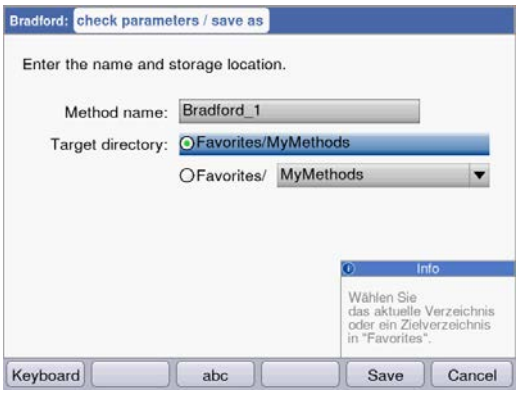
- [Page dn] and [Page up]: Change between 1 to 3 parameter list pages.
- [Edit]: Switch to the parameter edit mode.



Editing mode for parameters:
Modified parameters are marked with a red star until the modification has been saved.

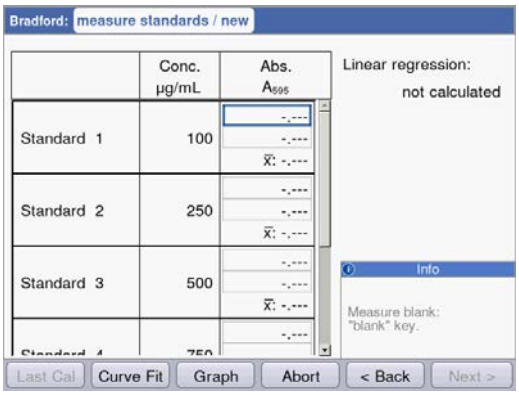
Softkeys

- [Save] and [Save as]: Save changes. When using [Save as] you have to rename the method. This is always the case when modifying the methods preprogrammed by Eppendorf in the ***Routine*** group.
- [Cancel]: Exit the edit mode without saving changes.



Saving the method under a new name:
You can save the method in the same folder from which you called up the method or in any folder in the ***Favorites*** method group.
The name (maximum 20 letters) can be entered using a displayed keyboard ([Keyboard] softkey) or directly using the keyboard (see *Entering text on p. 23)*.
After saving you will return to the **check parameters** display.

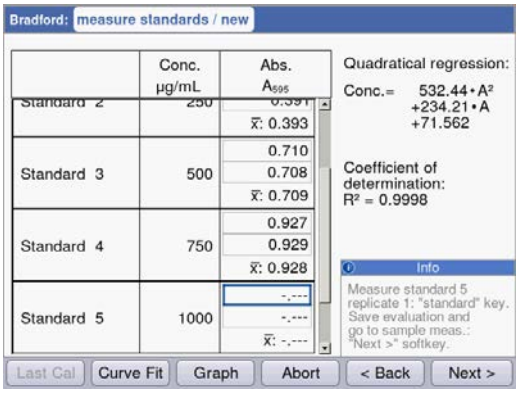
6.4.2 Measure standards



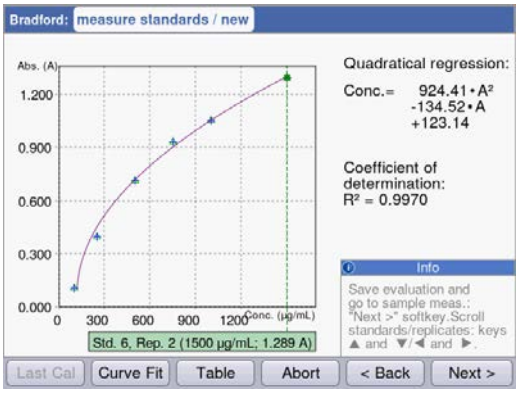
The first standard to be measured is marked on the display. After the blank value (**blank** key) measure all standards (**standard** key) one by one.
When measuring more than one replicate per standard, the average value for each standard is calculated and displayed automatically.
By using the ▲ and ▼ cursor keys, you also can select certain standards for measurement. Individual standards can be remeasured as well.

Softkeys

- [Last cal]: Call up the last saved standard evaluation for this method in order to use it for sample measurements.
- [Curve fit]: Select the procedure for the standard evaluation. If the result has not been saved, the method can also be entered later. Instructions for selecting the evaluation procedure can be found in the Evaluation procedure chapter (see *Evaluation with standard curve/line on p. 106)*.
- [Graph]: Switch to the graphic display of the standard results.



As soon as the minimum number of results for the evaluation with the selected method (curve fit) is available, the evaluation result will be shown on the right side of the display. You can now save the evaluation and switch to sample measurements via the [Next >] key.



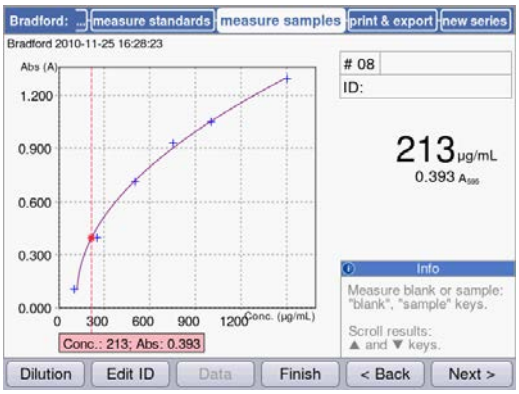
Graphical view of the standard evaluation.
With the ◄ and ► cursor keys, you navigate between the standards to view the results. With more than one replicate per standard, you can switch between the replicate results using ▲ and ▼. You can also select individual standards from the graphical display and measure or remeasure them.

Softkeys

- [Table]: Switch to the table display of the standard results.
- [Next >]: Save the standard evaluation and switch to the sample measurement.

6.4.3 Measure samples

The **sample** key is used for measuring your samples consecutively. Blank results remain saved for one measuring series, but a new blank result measurement can be performed at any time. With the ▲ and ▼ keys you can navigate between the sample results that have been achieved in the measuring series up to this point.



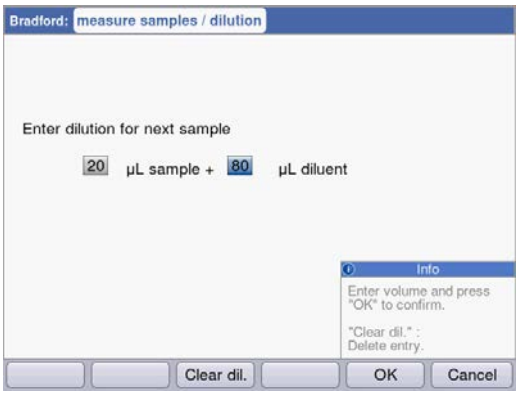
Results display:
- The concentration result (6 digits with floating point) is clearly emphasized.
- With graphic: Result to the right of the display.
- Without graphic: Result in the middle of the display.
- In addition to the result, the basic absorbance value is shown at a smaller scale.

Additional data
- Upper right; first row:
  Sample number: Counted sequentially and reset to "1" for each new series of measurements.
  Sample dilution (if provided)
- Upper right; second row:
  Sample identification (**ID**) (if provided)
- Top left:
  File name with which the data in the **print and export** method step can be exported as Excel file (see p. 62).

Softkeys
- [Dilution]: Enter sample dilution.
- [Edit ID]: Enter sample ID
- [Data]: Display additional result data (not available with all methods).
- [Finish]: End series of measurements and return to method selection.

The displayed absorbance values always correspond to the directly measured values. The dilution or cuvette factor as well as background absorbances will be incorporated for the following result calculation (see *Absorbance values on p. 103)*.

Enter dilution



The [Dilution] softkey is activated after the blank (**blank** key) has been measured.

1. Press the [Dilution] softkey.
2. Enter the volumes for the sample (up to 3 digits) and for the dilution buffer (up to 4 digits).

The device will multiply the following sample results by the calculated dilution factor.

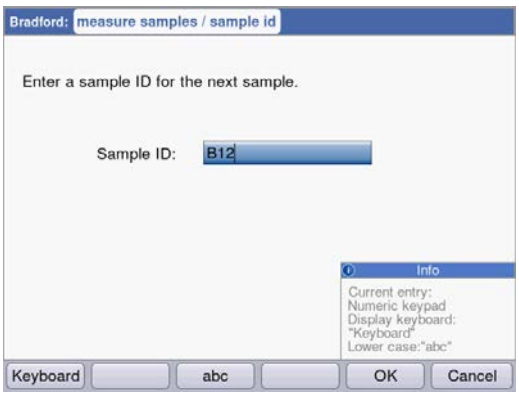
Softkeys

- [Clear dil.]: Delete values for sample dilution.
- [OK]: Confirm sample dilution and return to sample measurement.
- [Cancel]: Cancel entry and return to sample measurement.

The dilution is used for all following sample results until it is changed by a new entry.

Enter sample ID

The ID will be applied to the following sample result. When an ID is being entered the last entered ID will be displayed as a default template to allow the quick entry of IDs with a consecutive structure. A single ID can only be assigned once for the same measuring series.



1. Press the [Edit ID] softkey.
2. Enter the sample ID (up to 12 digits).

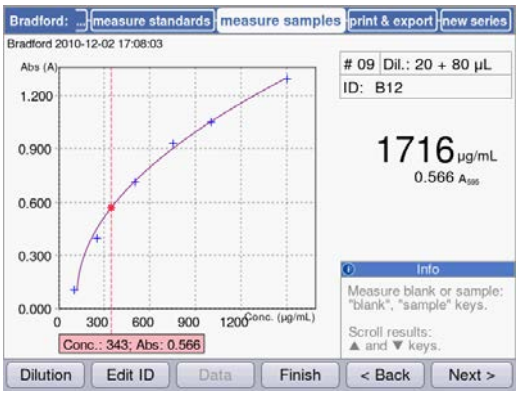
Alternatives for character input:

- Keypad: If the key is pressed several times in a row, the possible entries for this key will be shown consecutively.
- Display keyboard with softkey [Keyboard]: Select character with the cursor keys and confirm with **enter**.

Softkeys

- [Keyboard]: Display keyboard.
- [abc]: Change between upper and lower case letters when making entries with the keypad.
- [OK]: Confirm ID entry and return to sample measurement.
- [Cancel]: Cancel entry and return to sample measurement.

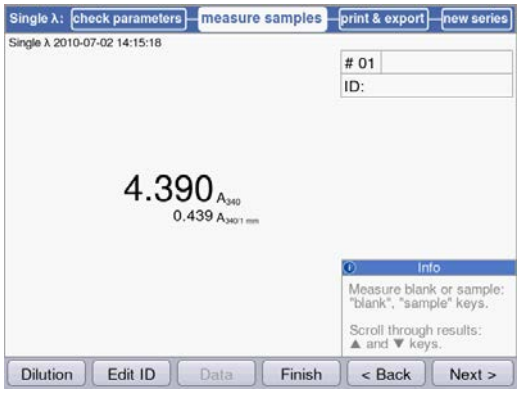
Result image with dilution and ID

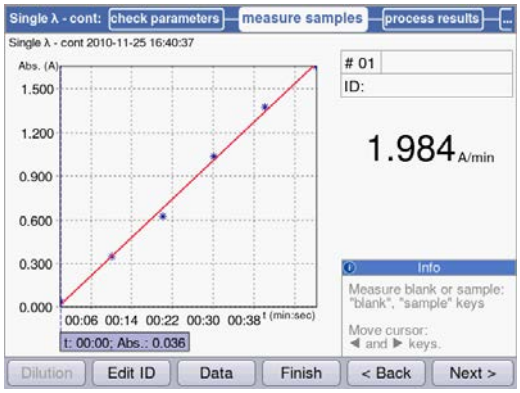
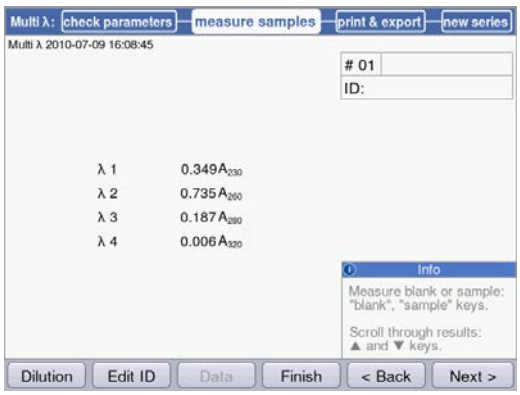


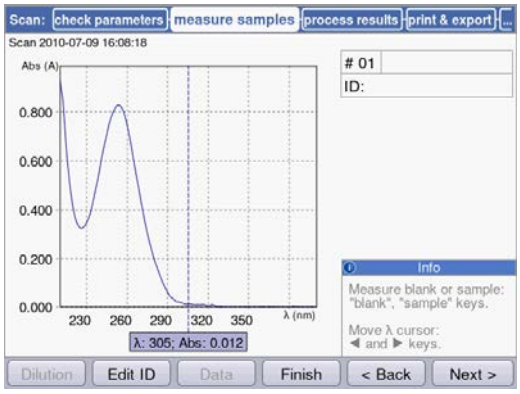
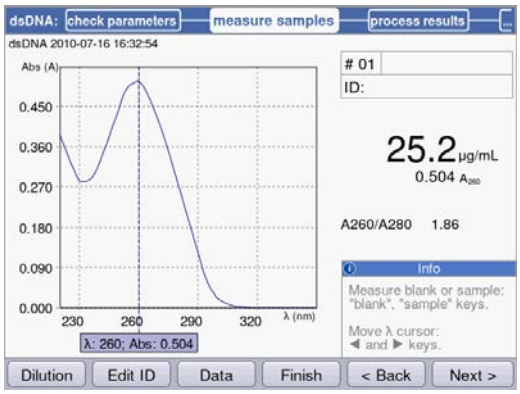
Result image with dilution and sample ID

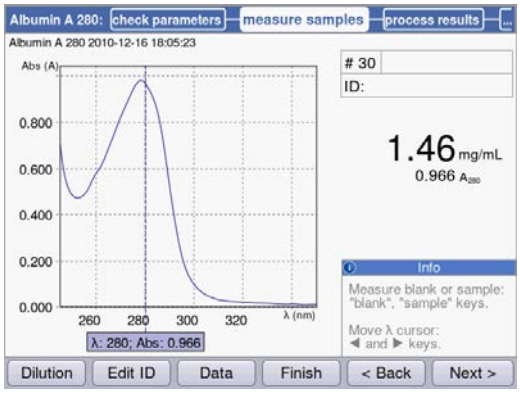
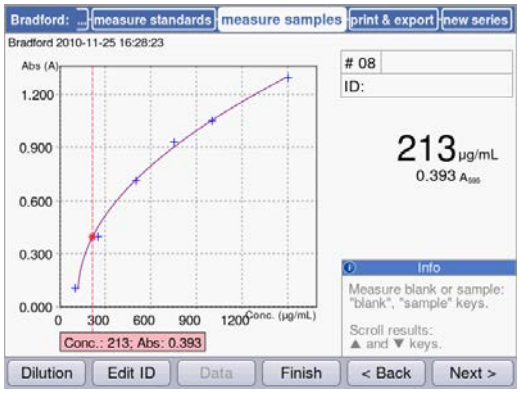
6.4.4 Measure samples: Results displays

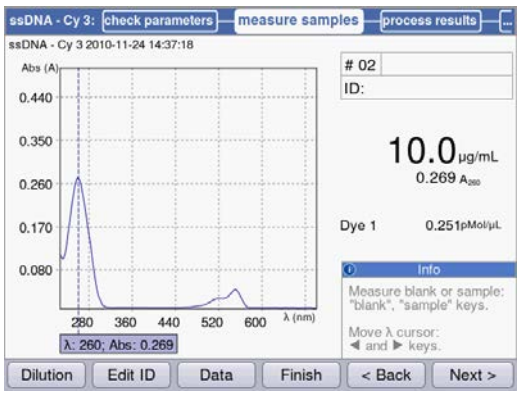
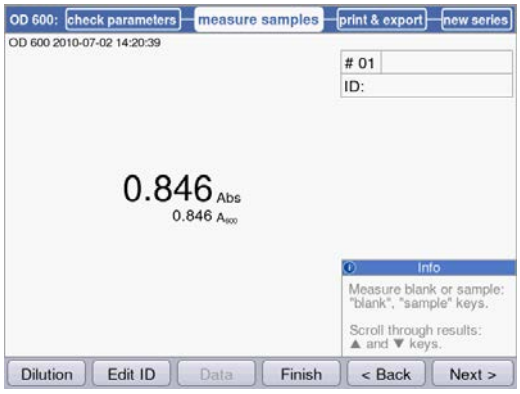
This section contains a display of typical results displays for all method groups and an overview of additional results data, which can be accessed using the [Data] softkey.

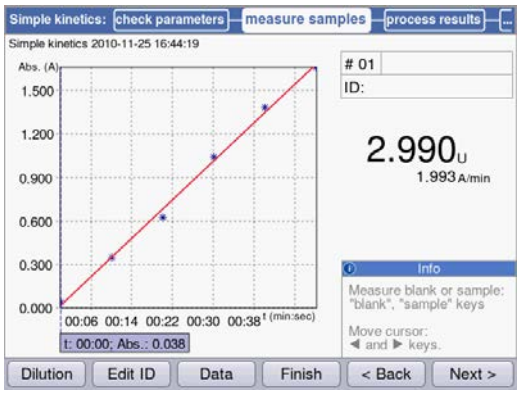
Method group	Results display	Explanation
***Absorbance* main group**		
Single λ		Results display: • Absorbance at the measuring wavelength • Only for dilution or with cuvettes other than 10 mm: additional display of the absorbance value before the conversion.

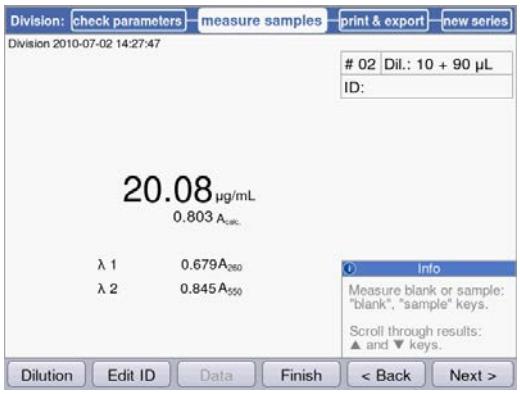
Method group	Results display	Explanation
Single λ - continuous		Prior to measurement: • **Check parameters** method step: Display temperature control. When the temperature set in the parameters has been reached and the display changes from **Tempering** to **Ready**, you can go to the measurement. During the measurement: • You can prematurely cancel the measurement using the [Stop] softkey. Results display: • Graph with absorbance time display and plotted regression line. • Value for "A/min" calculated from the linear regression. Additional data ([Data] softkey): • Absorbance time value pairs for the first and last measuring points. • Quality parameters for linear regression • Navigate between the measuring points in the graph with ◄ and ►. • If necessary, change the time frame for evaluation with linear regression in the **process results** method step.
Multi λ		Results display: • Absorbance values at the wavelengths Additional data ([Data] softkey): • Only for dilution or with cuvettes other than 10 mm: absorbance value before the conversion.

Method group	Results display	Explanation
Scan		Results display: • Scan (graph with absorbance wavelength display) • Navigate between the measuring points in the graph with ◄ and ►.
***Routine* main group**		
Nucleic acids		Results display: • Concentration result with absorbance at the measuring wavelength • If activated in the parameters: Ratios A260/A280 • If activated in the parameters: Scan. Navigate between the measuring points on the graph which are used for the result calculation with ◄ and ►. Additional data ([Data] softkey):. If the corresponding parameters have been activated: • Absorbance value for 280 nm. • Ratios A260/A230 and absorbance value for 230 nm. • Absorbance value for the background wavelength.

Method group	Results display	Explanation
Proteins direct UV		Results display: • Concentration result with absorbance at the measuring wavelength • If activated in the parameters: Scan. Navigate between the measuring points on the graph which are used for the result calculation with ◀ and ▶. Additional data ([Data] softkey):. If the corresponding parameters have been activated: • Absorbance value for 260 nm. • Absorbance value for the background wavelength.
Proteins (with reagent)		Results display: • Concentration result with absorbance at the measuring wavelength. • For evaluation with standard series: graph of the standard evaluation with plotted sample result.

Method group	Results display	Explanation
Dye labels		Results display: • Concentration results with absorbance at the measuring wavelength of the biomolecule. • If activated in the parameters: Scan. Navigate between the measuring points in the graph with ◄ and ►. Additional data ([Data] softkey):. If the corresponding parameters have been activated: • Ratios A260/A280 and A260/A230. • Absorbance values for 280 nm and 230 nm and for the measuring wavelength of the dye. • FOI value. • Absorbance values for the background wavelengths. Ratios and FOI are not displayed for the measurement of the dye-labeled proteins.
Bacterial density		Results display: • Calculated result with absorbance at the measuring wavelength. • If activated in the parameters: Scan. Navigate between the measuring points in the graph with ◄ and ►.

Method group	Results display	Explanation
***Basic* main group**		
Factor, standard	Analog to *Protein direct UV* (see above)	Results display: • Concentration result with absorbance at the measuring wavelength.
Calibration curve	Analog to *Proteins (with reagent)* (see above)	Results display: • Concentration result with absorbance at the measuring wavelength. • Graph of the standard evaluation with plotted sample result.
Simple kinetics		Prior to measurement: • **Check parameters** method step: Display temperature control. When the temperature set in the parameters has been reached and the display changes from **Tempering** to **Ready**, you can go to the measurement. Results display: • Concentration result with absorbance or A/min with the measuring wavelength. • Only for the "linear regression" measuring procedure: graph with absorbance time display and plotted regression line. Navigate between the measuring points in the graph with ◄ and ►. Additional data ([Data] softkey):. Dependent on the measuring procedure: • Absorbance time value pairs for the first and last measuring points ("endpoint" method: only one measuring point). • Quality parameters for linear regression (omitted for kinetics with a slope below 0.050 E/min).

Method group	Results display	Explanation
***Advanced* main group**		
Dual wavelength		Results display: • Concentration result: calculated from $\mathbf{A_{calc.}}$ with factor or standard evaluation. • $\mathbf{A_{calc.}}$: calculated using the formula, defined in the parameters, created from the absorbances measured on both wavelengths. • Absorbance values that were measured at the two measuring wavelengths. Additional data ([Data] softkey):. If the corresponding parameters have been activated: • Absorbance value for the background wavelength.
Advanced kinetics	Analog to *Simple kinetics* (see above).	In addition to the data similar to the **Simple kinetics** method group: Additional data ([Data] softkey):. If the corresponding parameter was activated: • Absorbance or A/min for the reagent blank.

6.4.5 Process results

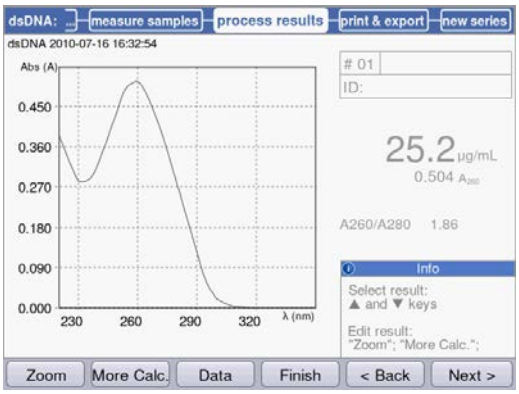
The sample measurement is followed by two optional steps in the method sequence: **process results** and **print & export**.

In the **process results** step, you can postprocess the results for some methods. Example: Changing the spectra section of a scan.

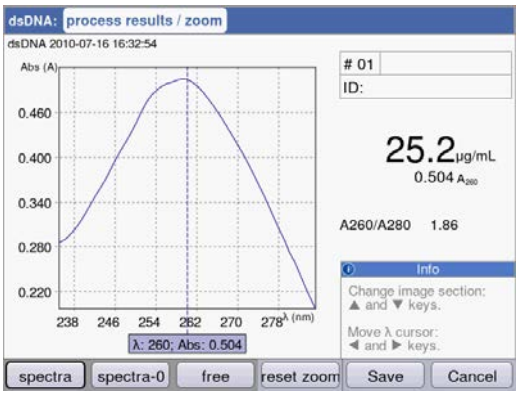
As for the result display, you can navigate between the sample results of the measurement series with the ▲ and ▼ cursor keys and select results for postprocessing.

Tab. 6-3: Options: Overview

Option	Explanation	Available in methods
Zoom	Change the axis limit of the absorbance wavelength graph to limit the view to enlarged sections of the graph.	Basically all the methods for which the **Scan** parameter is available and has been activated. • **Multi λ** • **Scan** • **Nucleic acids** • **Proteins direct UV** • **Dye labels**
More calculations	Convert concentration results into molar concentrations and (after entering the volume) into total amounts.	• **Nucleic acids** • **Dye labels** (with nucleic acids as biomolecule)
Peak detection	Identifying peaks in absorbance wavelength spectra.	• **Scan**
Linear regression	Modifying time windows for the evaluation of a kinetic via linear regression.	In principle, all kinetics methods for which the "Linear regression" measuring procedure was used. • **Single λ - continuous** • **Simple kinetics** • **Advanced kinetics**

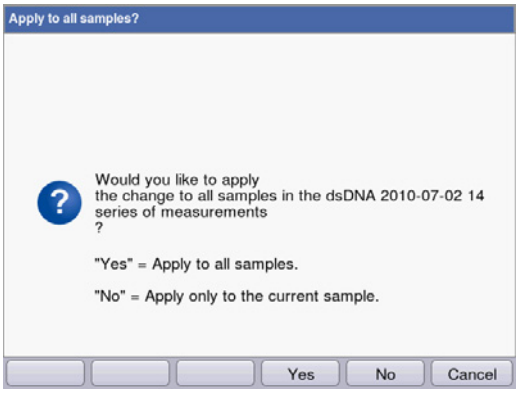


Options for postprocessing are presented on the two softkeys at left. In this example: [Zoom] and [More Calculations].



After changes have been made, you can exit the current mode using the two softkeys at right:

- [Save]: Save changes and return to the **process results** method step.
- [Cancel]: Cancel and return to the **process results** method step.

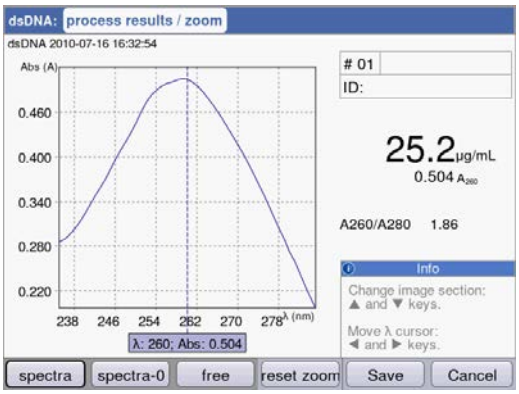


After the changes have been saved you can apply them to all samples of the measuring series with [Yes].

6.4.6 Process results: Options

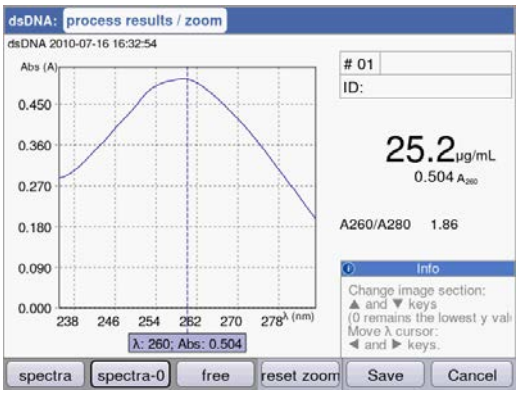
Zoom

Press the [Zoom] softkey and select one of the following versions.

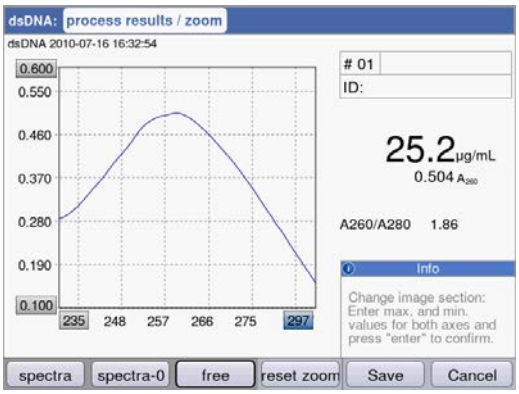


Variant [spectra]:

- Cursor keys ◀ and ▶: Move the wavelength cursor. It determines the zoom center above the x-axis.
- Cursor keys ▲ and ▼: Gradually zoom in and out of the displayed section of the x-axis using the SpectraZoom procedure.
  The shown section of the y-axis is automatically adjusted with each step so that maximum and minimum of the data to be displayed make optimum use of the section.



Variant [spectra-0]:
Same as the [spectra] variant, with one exception:
The lower limit of the displayed section of the y-axis always equals "0 A".

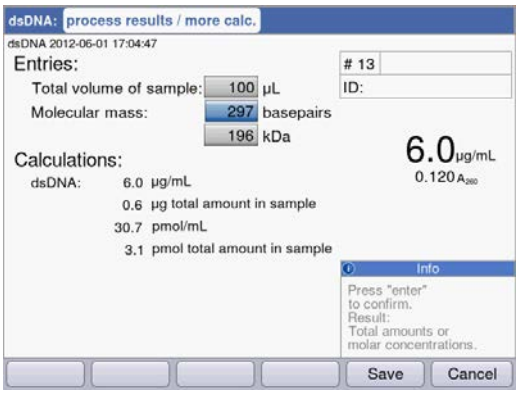


Variant [free]:
User-defined values for interval limits can be entered for both axes.
Navigate between the entry fields using the cursor keys (▲, ▼ ◀, ▶).

For all 3 versions, the [reset zoom] softkey will bring you back to the original display of the spectrum.

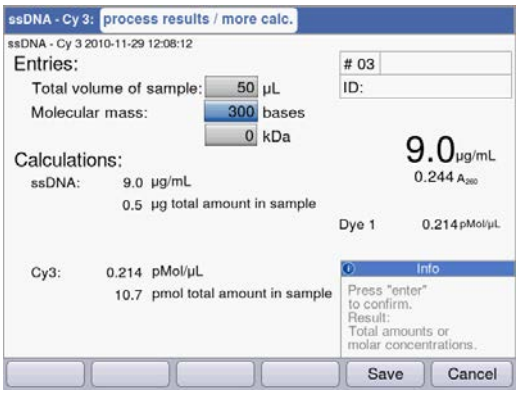
More calculations

Press the [More calc.] softkey.



Nucleic acids method group:

- After the molar mass has been entered (in base/ base pairs or in kDa): Convert the concentration result to the molar concentration.
- After the sample volume has been entered: Calculate the total amount in the sample.



Dye labels method group:
Nucleic acid:

- After the molar mass has been entered (in base/ base pairs or in kDa): Convert the concentration result to the molar concentration.
- After the sample volume has been entered: Calculate the total amount in the sample.

Dye:

- After entering the volume of the sample: Calculate the total amount in the sample.

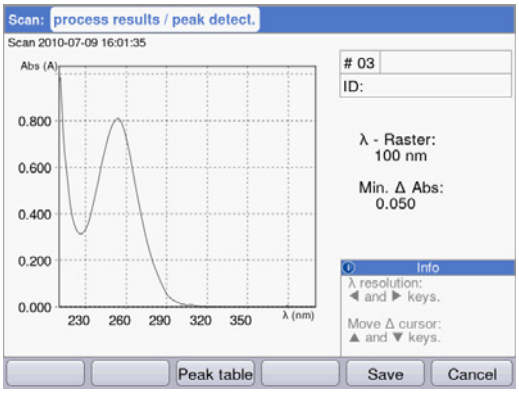
- For **dsDNA** the calculation of the molar concentration is based on the assumption of a double-stranded nucleic acid. For the **ssDNA**, **RNA** and **Oligo** methods, a single-stranded nucleic acid is assumed.
- For methods which have been reprogrammed via **<New Method>** in the ***Routine*** main group, **Nucleic acids** method group, always double-stranded nucleic acids are assumed for calculating the molar concentration.

Peak detection

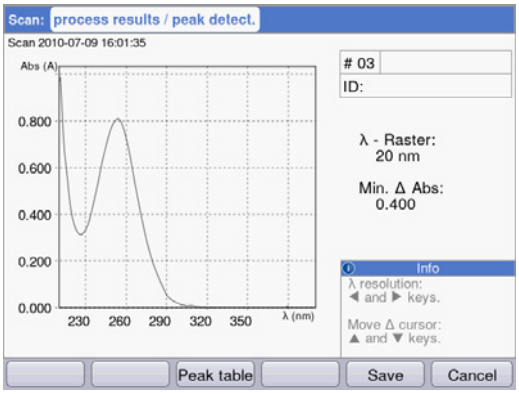
Press the [Peaks] softkey. For the peak detection you can alternate between two criteria:

- **λ grid**: Evaluation grid on the wavelength scale for the peak detection (e.g., 10 nm).
  10 nm example: The spectra section from -5 nm to +5 nm is evaluated in relation to the peak to be detected.
- **Min. Δ Abs**: Minimum difference between the peak to be detected and the lowest absorbance in the evaluation grid. No absorbance value in the grid may be higher than the value of the peak at any given time (e.g.: 0.5).

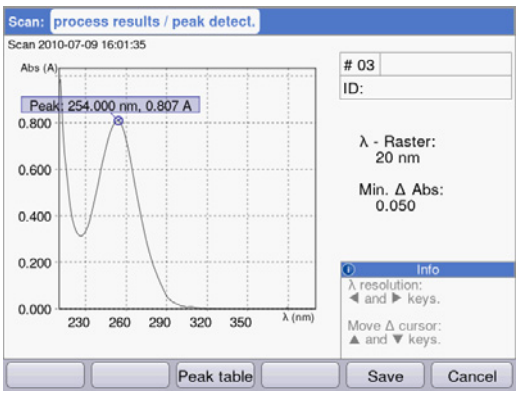
Examples:



λ grid: 100 nm, min. Δ Abs: 0.050:
The peak is not detected because the λ grid is too large: The absorbance values on the left edge of the grid are higher than the absorbance of the peak.



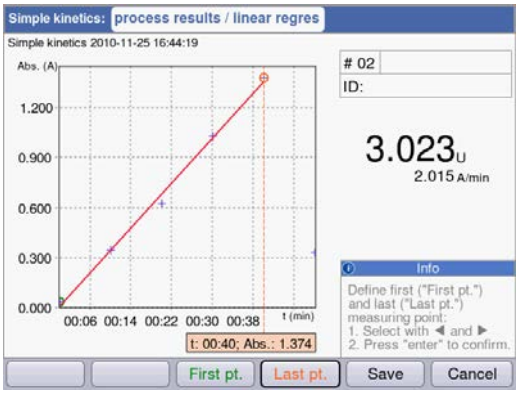
λ grid: 20 nm, min. Δ Abs: 0.200:
The peak is not detected because the predetermined value for **min. Δ abs** is too high. The difference of the absorbance of the peak and the lowest absorbance in the grid is less than 0.2 A.



λ grid: 20 nm, min. Δ Abs: 0.050:
The peak is detected.

Linear regression

For kinetic methods evaluated using the "Linear regression" measurement method, you can subsequently define the start and end times for the regression evaluation in the **process results** method step.



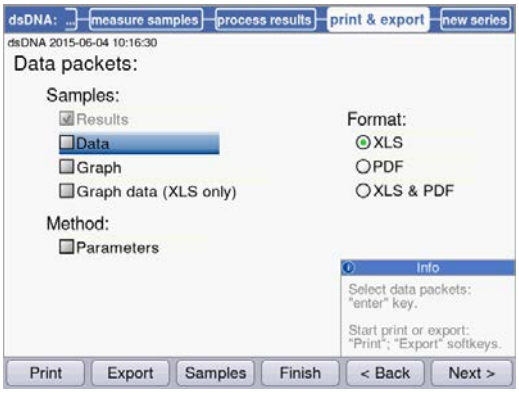
Selecting measuring points

- [First pt.] softkey: Define the first measuring point for the regression evaluation. Select the measuring point using ◄ and ►. Confirm with **enter**.
- [Last pt.] softkey: Define the last measuring point for the regression evaluation. Select the measuring point using ◄ and ►. Confirm with **enter**.
- At the same time that the times are modified, the result is recalculated. The reagent blank is also recalculated in the newly defined time window for this purpose.

6.4.7 Print & export

In the last optional method step you can compile data packages for all or selected samples of a series of measurements:

- for printing on the printer
- for exporting to a USB stick
- for exporting directly to a PC via a USB cable
- for exporting by e-mail



Select data packets
- Use the cursor keys for navigating and confirm with **enter**.

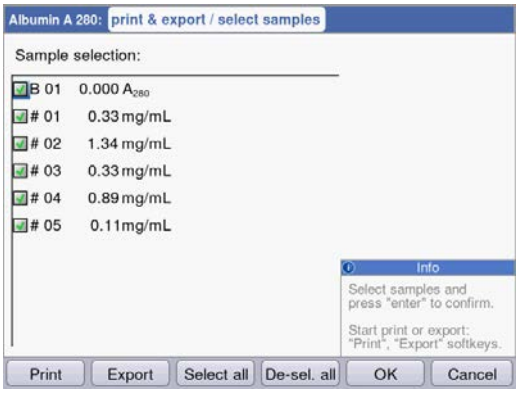
Select format
- XLS: Export as an Excel spreadsheet or print.
- PDF: Export as a PDF or print.

Softkeys
- [Print]: Start printing.
- [Export]: Start export.
- [Sample]: Select individual sample results.

Select data packets	
Results	Primary result data; cannot be selected because they are always transferred.
Data	Additional results data that are displayed during the measurement using the [Data] softkey.
Graph	Absorbance-wavelength-spectrum. (Only for kinetic methods with the "linear regression" measuring procedure: absorbance time graph.)
Graph data	The basic numeric data for the graph. "export only": Only available for export, i.e., not for printing.
Parameters	Method parameters
Standards/results	Results data of the standard evaluation.
Standards/graph	(Only for standard evaluations with several standards:) absorbance concentration graph.

Based on the method and parameter setting, only the available data packets are presented.

Select individual sample results



Select samples

- Press the [Samples] softkey to call up the sample selection.
- Use the cursor keys for navigating and confirm with **enter**.

Softkeys

- [Select all]: Select all samples
- [De-Sel. all]: Cancel selection.

Start export

The data will be transferred as an Excel file (.xls) or as a PDF. Excel files can be read with Excel versions Excel 97 and later. For each of the selected data packets, a worksheet is created in Excel. The file name consists of the method name, the time and the date of the measuring series.

Select export version

- Use the cursor keys for navigating and confirm with **enter**.
- Export to external storage medium: Save data to a USB stick.
  If no USB stick is connected, this option cannot be selected.
- Export to PC: Save data to a PC.
- Export via email: Send the data to an e-mail address.

Export to USB stick

1. Connect to FAT 32-formatted USB stick to USB port **4** (see *Main illustration on p. 15)*.
2. Use [Export] to start the "Export to an external storage medium".

Export to PC

Requirement for the PC operating system: Windows XP, SP2 or a later version.

1. Connect the device to the PC using the USB cable on USB port **8** (see *Main illustration on p. 15)*.
2. Prior to beginning a new export make sure that any data that has been exported previously has been saved to the PC hard drive. Otherwise, the new export will overwrite the data.
3. Use [Export] to start “Export to PC".
4. The exported data packet will be displayed on your PC as a removable medium named "eppendorf". Open the file on this drive and save it to the hard disk.

Export to an e-mail address

1. Select an e-mail address or select “Edit” to set up a new e-mail address.
2. Use [Export] to start “Send to an e-mail address”.

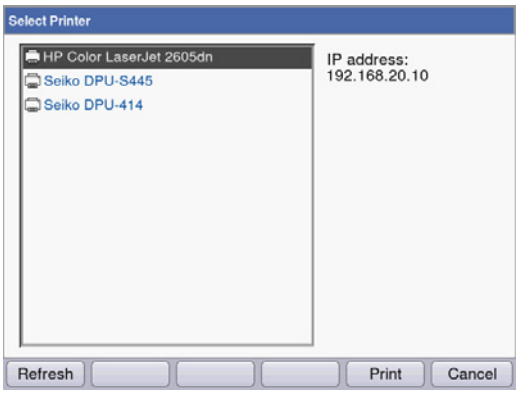
Editing e-mail addresses

- Select "Edit" from the drop-down list and confirm with **enter**.
  This will open a window in which the e-mail addresses can be edited.
- [Edit]: Editing e-mail addresses.
- [New]: Setting up a new e-mail address.
- [Delete]: Deleting e-mail addresses.

Start printing

The dasta can be printed via network printers or via a USB printer that has been connected.

If the device is connected to a network, all compatible printers in the network are recognized and displayed automatically. If there is no connection to the network, only a connected USB printer will be available.

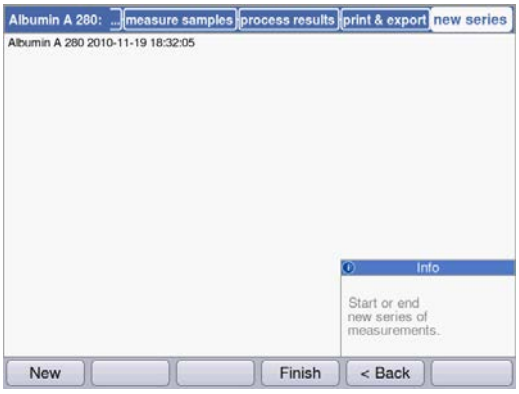


1. Select a printer.
2. Use [Print] to start printing the data.

6.4.8 Finish the series of measurements

After the **print & export** method step has been finished, you can start a new series of measurements using the selected method or select a new method.

Finish the series of measurements and start a new series of measurements



- [Next >] softkey: Call up **new series** method step
- [New] softkey: Call up **measure samples** method step and start a new series of measurements.

Finish the series of measurements and select a new method

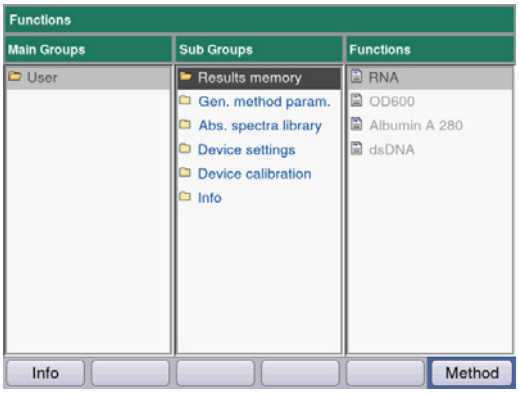
- [Finish] softkey: Close the series of measurements and call up the method selection.

7 Functions

7.1 Functions of the *User* main group

With the **function** key or the [Function] softkey, you reach a menu containing functions like device settings or calling up saved results.

The functions are structured in 3 columns analog to the method selection. The functions in the ***User*** main group are accessible to you. As for the method selection, you navigate with the cursor keys for selecting first the desired subgroup and then the desired function in the right column. Press **enter** to open the function.

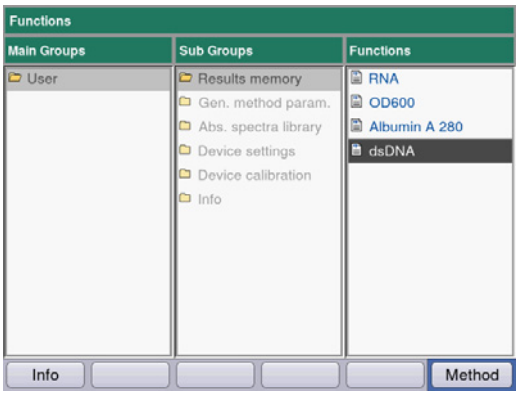


[Info] softkey: call up the firmware version and serial number of the BioSpectrometer kinetic.

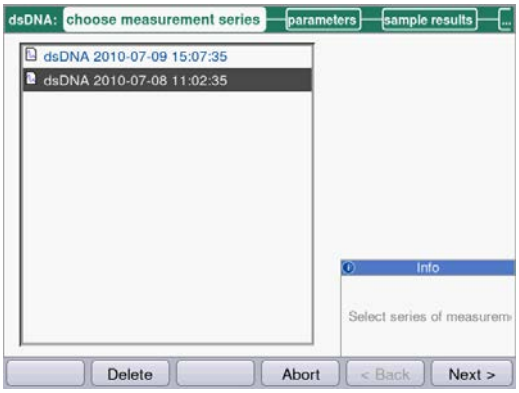
Tab. 7-1: Overview of the functions

Subgroup	Explanation
Results memory	Displays saved results. The results can be accessed structured according to methods and measuring series and can be printed and exported directly from the memory.
General method parameters	Parameters which are used for different methods in common are stored centrally in the **Functions** area. Here it is possible to edit (change or create anew) these parameters. In the **Check parameters** method step, the comprehensive parameters can be easily selected using drop-down menus. • **Proteins**, **nucleic acids**, **dyes** include parameters that are directly applied for methods of the **Dye labels** and **Proteins direct UV** groups. • **Units**: Units for concentration results which can be used for many methods.
Absorbance spectra library	Absorbance wavelength spectra of important substances, e.g., DNA. The spectra serve as information and can be used for comparison to a spectrum of a sample result.
Device settings	Editable device settings, e.g., language.
Device calibration	• Option for checking the spectrophotometer; An Eppendorf filter set is required for this. • Option for checking the thermal module.
Info	Open Source Licenses and information on registered trademarks.

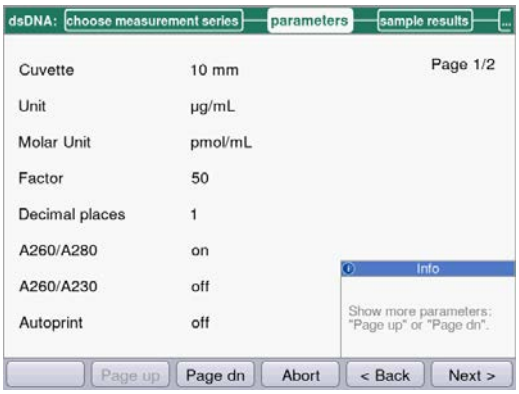
7.1.1 Results memory



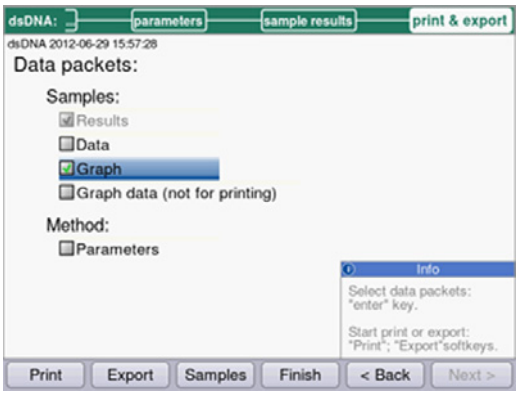
- In the right column, select the method for which you would like to call up saved results.
- Confirm with **enter**.



- Select the desired series of measurement with the cursor keys.
- Confirm with **enter**.



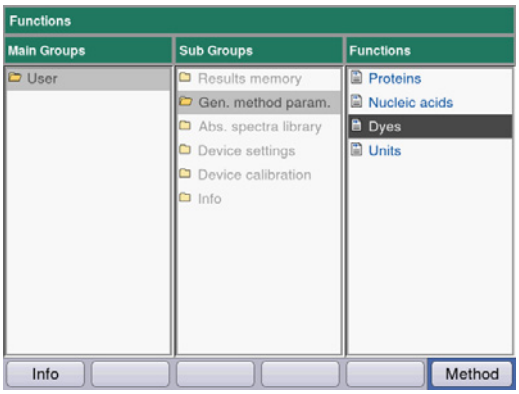
As in the method procedure, you can also successively switch between the display of the parameters, standards, sample results and, finally, the data packets for print and export.
The assignment of the softkeys matches the assignment in the method procedure.



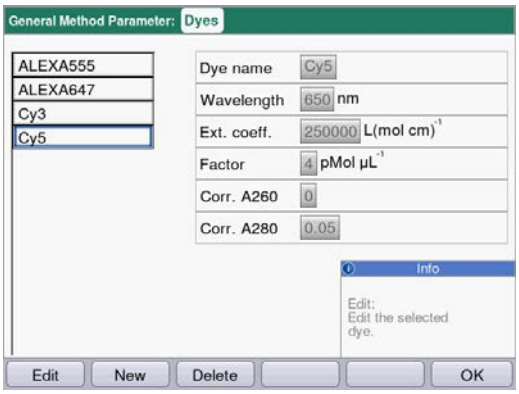
- If you would like to print or export results, select the data packets.

The procedure for printing and exporting and the meaning of the function keys corresponds to the **print & export** method step.

7.1.2 General method parameters



- In the right column, select the parameter group for which you would like to edit parameters.
- Confirm with **enter**.



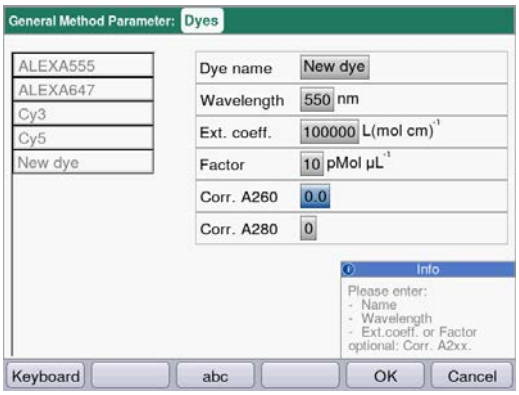
In this example, parameter groups are summarized for various dyes (dye components for the dye methods) and stored under a name. With this name, the required parameter group can be imported into the method program during the editing of a dye method.

Display:

- Left: Name of the dye. Select via ▲ and ▼.
- Right: corresponding parameters

Softkeys

- [Edit]: Edit selected parameter group.
- [New]: Create new parameter group.
- [Delete]: Delete selected parameter group.
- [OK]: Return to the function selection.

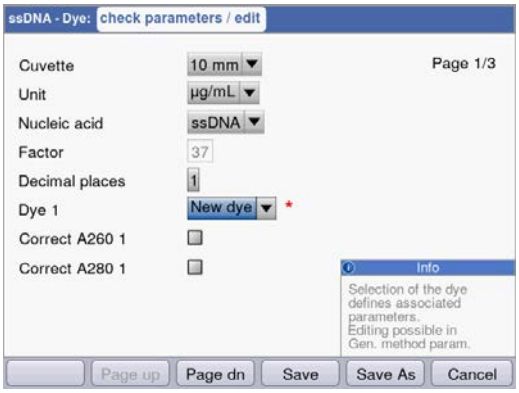


- To edit a parameter group, use ▲ and ▼ to select the parameter which you would like to edit.
- Confirm with **enter**.

Softkeys

- [OK]: Save entry and return to the parameter group selection.
- [Cancel]: Return to the parameter group selection without making any changes.

When programming a method of the **Dye labels** or **Proteins direct UV** method groups, you can access the entries in **General Method Parameter**:



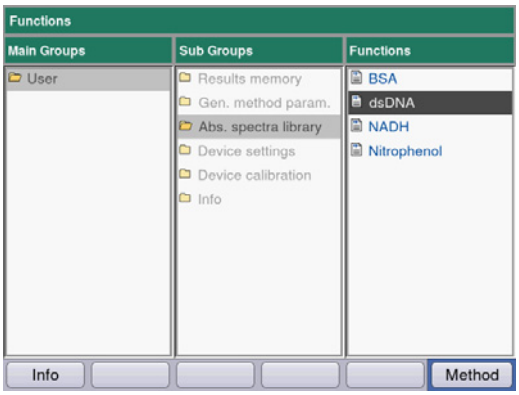
Select the name of the dye to import the corresponding parameter group into the method program. By using the "edit" selection of the "Nucleic acid" parameter, you also can get directly to the **General Method Parameter** function and view and edit the parameters.

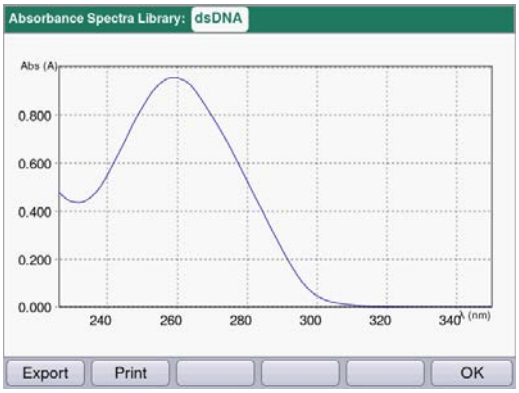
Tab. 7-2: Parameter in General Method Parameter

Parameter	Explanation
Proteins	These parameters are loaded into the method parameters when a protein is selected during the programming of a method in the **Dye labels** and **Proteins direct UV** groups.
• Protein name • Factor • $A_{0.1\%}$ • Ext.coeff. • Molecular mass	In order to define a factor for calculating the concentration on the basis of the absorbance, enter the following data in addition to the name and wavelength: Factor **or** $A_{0.1\%}$ **or** absorbance coefficient and molar mass.
Nucleic acids	These parameters are loaded into the method parameters when a nucleic acid is selected during the programming of a method in the **Dye labels** group.
• NA name • Factor • Double-stranded	The factor is used to calculate the concentration on the basis of the absorbance. The double-stranded parameter affects the calculation of the molar nucleic acid concentration. (see *Conversion to molar concentrations and nucleic acid quantities on p. 108)*
Dyes	These parameters are loaded into the method parameters when a dye is selected during the programming of a method in the **Dye labels** group.
• Dye name • Wavelength • Ext.coeff. • Factor • Corr. A260 • Corr. A280	In order to define a factor for calculating the concentration on the basis of the absorbance, enter the following data in addition to the name: Factor **or** absorbance coefficient. The correction factors for the absorbance values at 260 or 280 nm are used when the correction function in the method parameters is active. For more details, refer to the chapter on evaluation (see *Correction A_{260} and correction A_{280} on p. 107)*.
Units	You can select a unit from all available units when programming method parameters.
• Unit	Entering a unit that has not yet been programmed for the concentration result.

- Specifications for proteins which are not preset at the factory can be determined in the expasy database: http://www.expasy.org/tools/protparam.html.
- A table with $A_{1\%}$ values for many proteins can also be found in: C.N.Pace et al., Protein Science (1995), 4: 2411–2423 (Table 5). The $A_{1\%}$ values must be multiplied by 0.1 to return the required $A_{0.1\%}$ values.

7.1.3 Absorbance spectra library



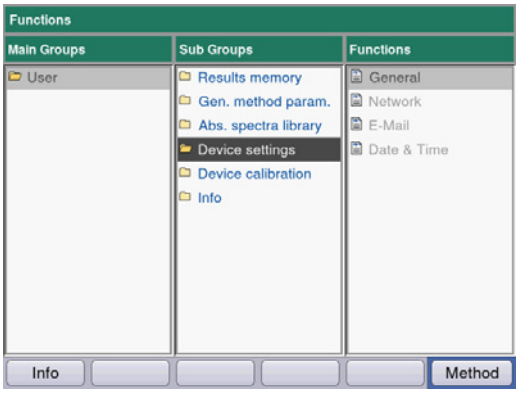


In the right column, you select the spectrum which you would like to call up and confirm with **enter**.

Softkeys

- [Export] and [Print]: Export to a USB stick or print to a PC using a USB cable (see *Print & export on p. 62)*.
- [OK]: Return to the function selection.

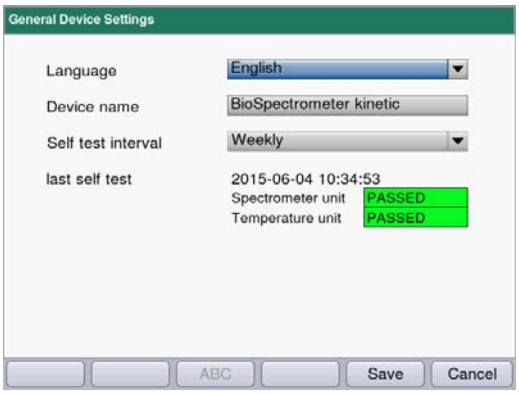
7.1.4 Device settings



The following settings can be adjusted:

Device Settings

- General
- Network
- E-Mail
- Date and Time

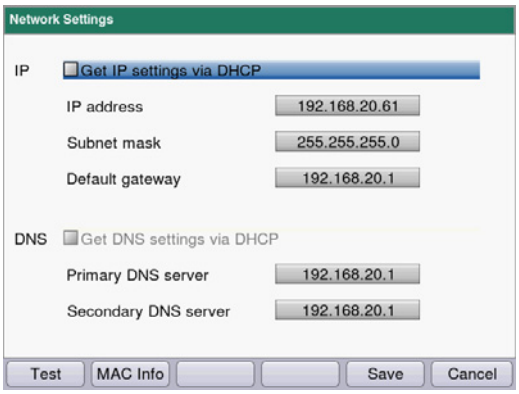


General Device Settings

- Select language: German, English, French, Spanish, Italian.
- Device name
- Setting the frequency of the automatic self test of the device after switching on.
- Information on the last self test is displayed.

Softkeys

- [Save]: Save changes and return to the function selection.
- [Cancel]: Return to the parameter group selection without making any changes.



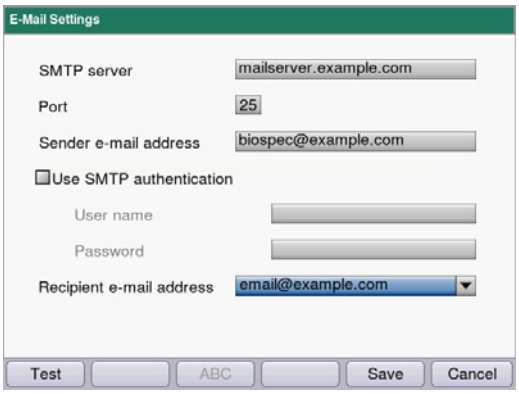
Network Settings

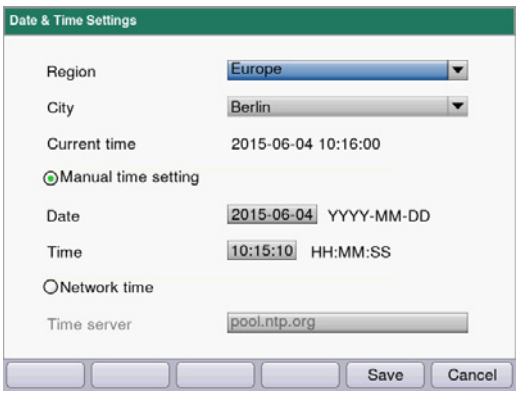
Ask your network administrator which settings are required.

- Select whether IP settings are to be made automatically via DHCP. The IP settings can also be entered manually.
  - IP-Address
  - Subnet mask
  - Standard gateway
- Select whether IP settings are to be made automatically via DHCP (only available if IP settings are received automatically via DHCP). The following DNS settings can be entered manually:
  - Primary DNS server
  - Secondary DNS server

Softkeys

- [MAC Info]: Information on network settings.
- [Save]: Save changes and return to the function selection.
- [Cancel]: Return to the parameter group selection without making any changes.





E-Mail Settings

Ask your network administrator which settings are required.

- SMTP Server: Enter the e-mail server.
- Enter the port.
- Sender: Enter the device name.
- Use SMTP authentication: If authentication is required, a user name and a password must be assigned.
- Recipient's e-mail address: List with e-mail addresses.

Editing e-mail addresses

- Select "Edit" from the drop-down list and confirm with **enter**.
  This will open a window in which the e-mail addresses can be edited.

Softkeys

- [Edit]: Editing e-mail addresses.
- [New]: Setting up new e-mail addresses.
- [Delete]: Deleting e-mail addresses.

Date and Time Settings

- Select the region.
- Select the town.
- Display of the current time
- Manual time setting: Enter the date and time.
- Network time
  Time server: Enter the required time server.

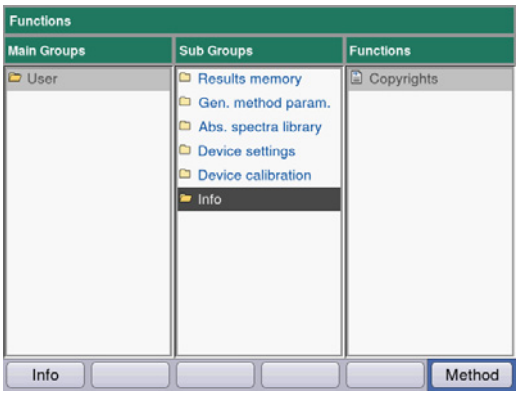
Softkeys

- [Save]: Save changes and return to the function selection.
- [Cancel]: Return to the parameter group selection without making any changes.

7.1.5 Device calibration

Information on checking the device is provided separately (see *Checking the device on p. 79)*.

7.1.6 Info



The **Copyright** menu item contains license information on the Open Source software.

8 Maintenance

8.1 Cleaning

DANGER! Electric shock as a result of penetration of liquid.

- Switch off the device and disconnect the power plug before starting cleaning or disinfection work.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not spray clean/spray disinfect the housing.
- Only plug the device back in if it is completely dry, both inside and outside.

NOTICE! Corrosion from aggressive cleaning agents and disinfectants.

- Do not use corrosive cleaning agents, aggressive solvents or abrasive polishes.
- Do not incubate the accessories in aggressive cleaning agents or disinfectants for a longer period of time.

1. Wipe down the surfaces with a cloth moistened with a mild cleaning agent.

Cleaning the cuvette shaft

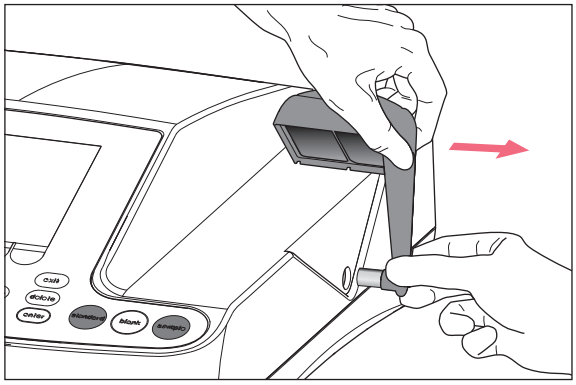
2. The cuvette shaft may only be cleaned with a lint-free cotton swab that has been dampened with ethanol or isopropanol. Prevent liquid from entering the cuvette shaft. If the shaft needed to be dampened with water to remove contamination, follow this up by cleaning the shaft with a cotton swab dampened with ethanol or isopropanol to accelerate the drying process.

8.1.1 Cleaning the cuvette shaft cover

If you would like not only to clean the directly accessible surface of the cuvette shaft cover, you can remove the cover.

- Do not soak the cuvette shaft cover in cleaning agent.
- Clean the cuvette shaft cover as described.

1. Lift the cuvette shaft cover with one hand.
2. With the other hand, hold the cover at the height of the locking pin and pull the cover to the right until the locking pin has been removed.



- Pull the cover to the right at a 90 degree angle.

3. Clean the cover with a cloth or lint-free cotton swab dampened with a mild cleaning agent.
4. Slide the locking pin back into the housing as far as it will go.

   The locking pin has completely disappeared in the housing.

When the photometer is not being used, close the cuvette shaft using the blue cuvette shaft cover to protect it from dust and other contamination.

8.2 Disinfection/Decontamination

DANGER! Electric shock as a result of penetration of liquid.

- Switch off the device and disconnect the power plug before starting cleaning or disinfection work.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not spray clean/spray disinfect the housing.
- Only plug the device back in if it is completely dry, both inside and outside.

1. Clean the device with a mild cleaning agent before the disinfection (see *Cleaning on p. 77)*.
2. Choose a disinfection method which corresponds to the legal regulations and guidelines in place for your range of application.
3. For example use alcohol (ethanol, isopropanol) or other alcoholic disinfectants.
4. Wipe the surfaces with a cloth which you have moisturized with a disinfectant.
5. If the cuvette shaft cover needs to be removed for the disinfection, proceed as follows for the diassembly and assembly (see *Cleaning the cuvette shaft cover on p. 78)*.
6. You can use spray disinfection to disinfect the disassembled cuvette shaft cover.

8.3 Checking the device

Prerequisites

- Observe the ambient conditions (see *Ambient conditions on p. 99)*.
- Perform the check at approx. 20 °C. Prevent temperature changes (e.g. due to open windows).
- Remove the filter from the filter box only for a short time and protect it against contamination or damages at the filter surfaces.
- Protect the filter against dust, heat, liquids and aggressive vapors.
- The filter is inserted in such a way that the label, with the name of the filter, points towards the detector. When the spectrometer unit is checked: label points forward.
- The cuvette shaft is free from contamination.

8.3.1 Checking the spectrometer unit

Eppendorf offers a filter kit (BioSpectrometer reference filter kit) for checking the photometric accuracy and wavelength systematic error. The kit contains one blank filter (A0) and three filters (A1, A2 and A3) for checking the photometric accuracy, and 3 filters for checking the wavelength systematic error in the range of 260 nm to 800 nm. The filter absorbances are measured in comparison with the blank filter A0. In addition to the information on the accuracy, you also receive information on the precision: From the respectively 15 measurements per wavelength the variation coefficient (cv-value) is determined next to the average value.

For measuring, first insert the blank filter (for the blank measurement) and then the test filter like cuvettes into the cuvette shaft. The absorbance values measured for the test filters are compared with the allowable value range. For the individual filters, the limit values for the permitted area are printed in a table in the lid of the filter box.

Please connect the Eppendorf thermal printer if you would like to document the values.

BioSpectrometer reference filter set **eppendorf**

Function : Device calibration/Spectrometer unit

Order No./Best. Nr.: 6135 928.001
Set No./Satz Nr.: 006

Limits measured against Blank A 0 at approx. 20°C Grenzwerte gemessen gegen Blank A 0 bei ca. 20°C							
SN: 6135	914.006	916.006	917.006	937.006	921.006	922.006	923.006
Filter Type	**Blank A 0**	**Sample 260 nm**	**Sample 280 nm**	**Sample 800 nm**	**Sample A 1**	**Sample A 2**	**Sample A 3**
Limiting values (A)/Grenzwerte (E)							
260 nm	0.000	1.291-1.499	--	--	0.197-0.221	1.078-1.101	1.857-1.888
280 nm	0.000	--	1.041-1.287	--	0.193-0.217	1.024-1.047	1.717-1.749
320 nm	0.000	--	--	--	0.173-0.196	0.923-0.947	1.478-1.509
405 nm	0.000	--	--	--	0.161-0.184	0.903-0.926	1.429-1.461
550 nm	0.000	--	--	--	0.172-0.195	0.930-0.959	1.487-1.526
562 nm	0.000	--	--	--	0.172-0.195	0.941-0.968	1.496-1.530
595 nm	0.000	--	--	--	0.172-0.195	0.943-0.969	1.503-1.538
700 nm	0.000	--	--	--	0.169-0.192	0.935-0.984	1.517-1.570
800 nm	0.000	--	--	0.958-1.124	0.168-0.191	0.931-0.983	1.502-1.561
		Random error of wavelength Zufällige Messabweichung der Wellenlänge			Random error of photometer Zufällige Messabweichung des Photometers		
Limiting values CV (%)/Grenzwerte VK (%)							
260 - 405 nm		≤ 3.0 %			≤ 3.0 %	≤ 2.0 %	≤ 1.5 %
550 - 800 nm		≤ 3.0 %			≤ 3.0 %	≤ 2.0 %	≤ 3.0 %

Wavelength and photometric accuracy/Wellenlängen- und photometrische Richtigkeit
traceable to/rückführbar auf
NIST SRM 2034, SN 04-A und SN 667

Wavelength and photometric characterization of filters:
All characterization is performed on a Cary 100 Bio reference UV-Vis spectrophotometer, SN EL 99023107.
The instrument is requalified regularly by the manufacturer, and is confirmed and documented to perform within manufacturer's specifications. The current instrument qualification is valid through April 2015.
For the current instrument qualification, the following, NIST traceable, certified secondary spectrometric calibration standards were used:

Wellenlängen- und photometrische Bestimmung der Filter:
Alle Messungen werden auf einem Cary 100 Bio Referenz-UV-Vis-Spektrophotometer, Seriennummer EL99023107 durchgeführt.
Dieses Instrument wird regelmäßig vom Hersteller requalifiziert und die spezifikationsgemäße Funktion dokumentiert.
Die aktuelle Qualifizierung ist bis April 2015 gültig und verwendet folgende, NIST-rückführbare und zertifizierte, spektrometrische Sekundärstandards:

Starna RM-0660HLKCSITX
Hellma 666-F1, 666-F2, 666-F3, 666-F4
SRM 2031a: SN 667, valid through/gültig bis 12/2014

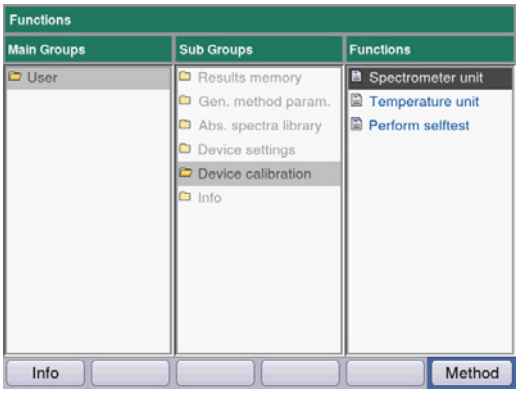
The valid certificates are part of the requalification documentation.
Die Gültigkeitszertifikate sind Teil der Qualifizierungsdokumentation.

6135 928.028-00

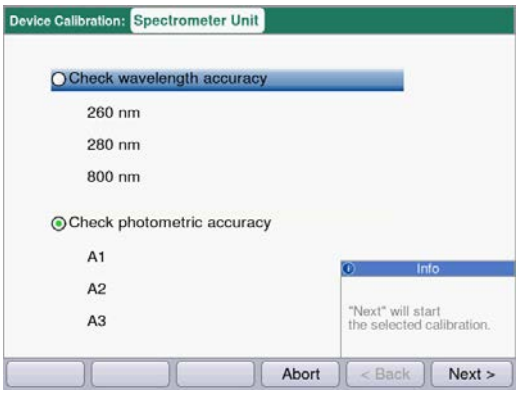
Please protect against dust, heat, cold and liquid. The limits are valid for max. 2 years. Bitte vor Staub, Hitze, Kälte und Flüssigkeiten schützen. Die Grenzwerte gelten für max. 2 Jahre.	Date Datum	Signature Unterschrift

Fig. 8-1: Inner lid of the filter box (model)

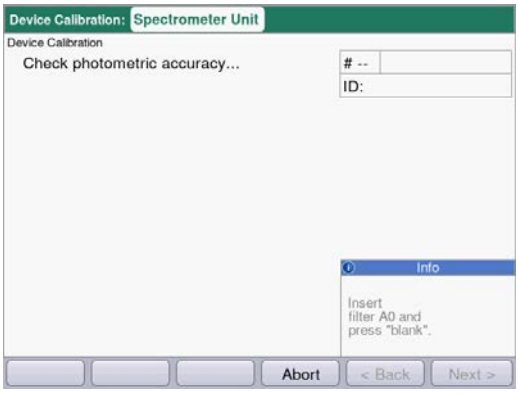
8.3.1.1 Checking the photometric accuracy



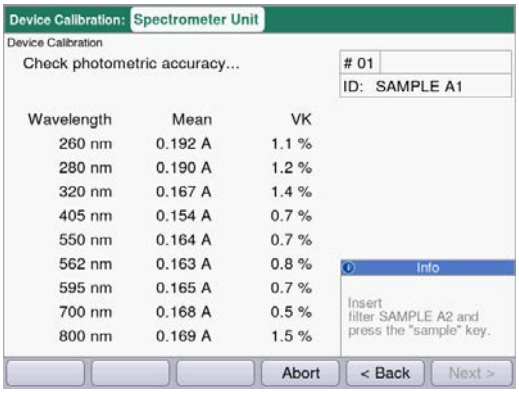
1. Select the **Spectrometer unit** function in the **Device calibration** group and confirm your selection with **enter.**



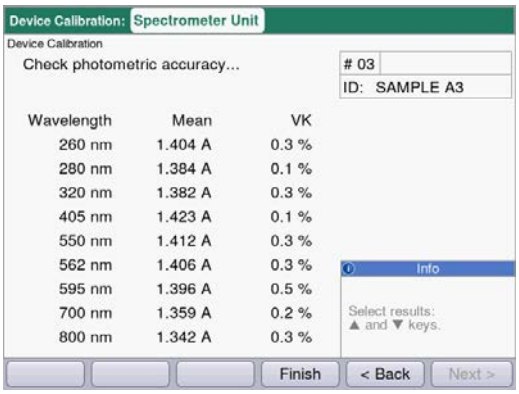
2. Select whether you want to check the wavelength systematic error or photometric accuracy, and confirm with **enter**. Press [Next >] to switch to the measurement.



3. Follow the instructions on the device display and start by measuring the A0 blank filter, and then the first test filter A1.
   The device measures the test filter 15 times at 9 wavelengths, and then shows the average value and the VC values for the series of measurements for all 9 wavelengths.



4. Result display after measuring a test filter for checking the photometric accuracy.
   Measure the other two test filters A2 and A3.



5. Results display after measuring all 3 test filters for testing the photometric accuracy.
   With the ▲ and ▼ keys, you can view again the results of the different test filters. Press [Finish] to complete the test.

6. Compare the average values and CV values to the supplied table.
   If the measured values do not agree with the permitted range of values, contact Eppendorf Service.

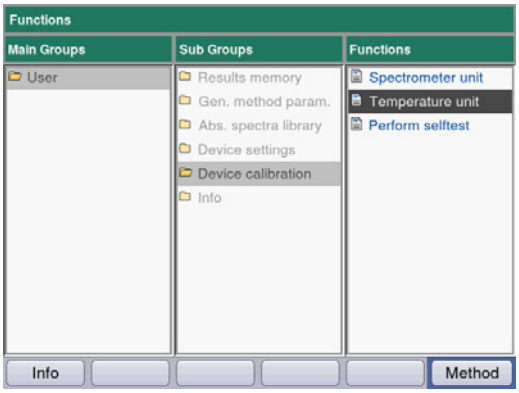
Have the filter recertified by Eppendorf Service after 2 years.

8.3.1.2 Checking the wavelength systematic error

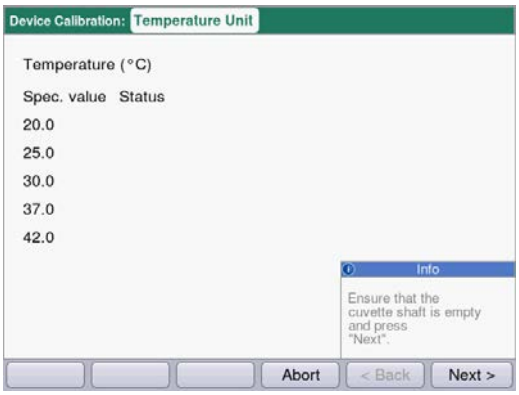
- To check the wavelength systematic error, proceed as follows: Measure the 3 test filters at the corresponding wavelength.

8.3.2 Checking the thermal module

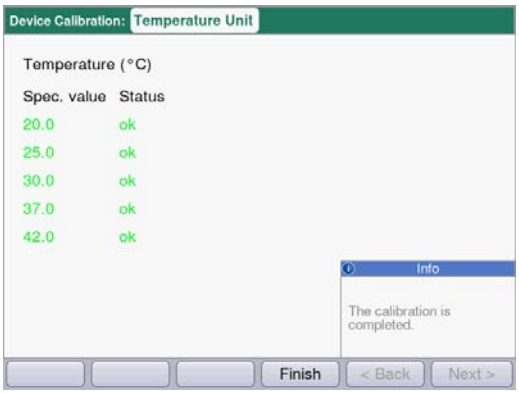
- Perform the check at approx. 20 °C.
- Make sure that the cuvette shaft is empty for the check.



1. Select the **Temperature unit** function in the **Device calibration** group and confirm with **enter**.



2. Make sure the cuvette holder is empty, and start the test procedure with [Next >].
   The following temperature control test at 5 temperatures takes approx. 30 minutes. The estimated remaining time is displayed during the test.



3. The results are displayed after the test has been completed.
   A positive test result is indicated by "OK" behind the temperature value. If the result "failed" is displayed for at least one temperature please contact Eppendorf Service.

8.3.3 Device self test

You can set the frequency of the automatic self test (taking approx. 1 minute) with the **Device settings** function (see *Device settings on p. 72)*. Ex-factory, the **self test interval** is set to "weekly".

The self test checks the following items:

- Verification of the detector
  - Determination of the random error across the entire available spectrum
- Verification of the light source
  - The device checks the maximally available light source energy and the light cable quality
  - Determination of the random error of a signal on the reference sensor
  - Determination of the signal level at the reference sensor
  - Separate determination of the light intensity in the UV range
- Determination of the systematic and random error of the wavelength
  - Position of an intensity peak in the UV range of the spectrum
  - Precision of the position of an intensity peak in the UV range of the spectrum
- Random error of the temperature sensor
- Verification of the temperature control rate

▸ In the **Device calibration** group, select the **Perform selftest** function and confirm with **enter.**

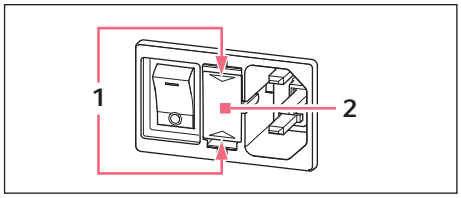
After the self test has passed, the display shows the message **PASSED**.
If the display shows the message **FAILED**, the self test has failed. If this error cannot be corrected (see *Error messages on p. 89)*, contact Eppendorf Service.

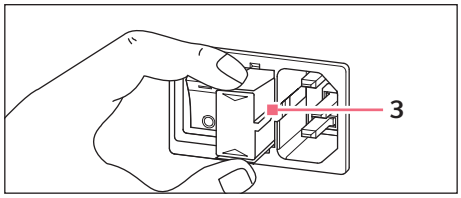
8.4 Replacing fuses

DANGER! Electric shock.

▸ Switch off the device and disconnect the power plug before starting maintenance or cleaning work.

The fuse holder is located between the mains connection socket and the mains power switch.





1. Disconnect the power plug.
2. Press the upper and lower end of the plastic springs **1** together and pull the fuse holder **2** fully out.
3. Replace faulty fuses and reinsert the fuse holder. Make sure that the guiding rail **3** is positioned correctly.

8.5 Decontamination before shipment

If you are shipping the device to the authorized Technical Service for repairs or to your authorized dealer for disposal please note the following:

WARNING! Risk to health from contaminated device

1. Follow the instructions in the decontamination certificate. You find it as a PDF file on our website (www.eppendorf.com/decontamination).
2. Decontaminate all the parts you would like to dispatch.
3. Include the fully completed decontamination certificate in the packing.

9 Troubleshooting

9.1 General errors

Error	Possible cause	Remedy
Measuring results are imprecise.	• Reagent is past its shelf life.	▸ Ensure that the reagent is still within its shelf life and properly prepared.
	• Reagent has not been prepared properly.	▸ If required, use clean demineralized water of an adequate quality for preparation.
	• The pipetting is not correct.	▸ Ensure that the pipette is calibrated and pipetting is being performed correctly.
	• Incubation procedure before measurement is incorrect.	▸ If the method procedure requires incubation before the measurement, ensure that the temperature and time for incubation are correctly observed.
	• The cuvette is contaminated.	▸ Clean and rinse the cuvette. When replacing a cuvette, pay attention that the optical window of the cuvette remains clean and that you do not touch it with your fingers. ▸ If the cuvette window has become soiled from fingerprints, wipe it clean using a lint-free lab cloth soaked in ethanol or isopropanol.
	• The cuvette is not filled completely with measuring solution, and it contains bubbles.	▸ Ensure that the required minimum volume of the cuvette for a measurement is reached and that no bubbles are in the measuring solution.
	• Turbidity of the measuring solution.	▸ Centrifuge the turbid measuring solutions that contain particles and use the clear supernatant.
	• Spectrophotometer is drifting.	▸ Contact Eppendorf Service. ▸ Observe the ambient conditions. ▸ Prevent temperature changes.
	• Cuvette shaft is dirty.	▸ Clean the cuvette shaft .

Error	Possible cause	Remedy
Measuring results are not correct.	• The method has not been programmed correctly.	▸ Ensure that the method parameters are entered correctly.
	• The standard solution has not been prepared correctly.	▸ Ensure that the correct standard is used and that the measuring solution for the standard is prepared correctly.
	• The absorbance of the reagent is drifting.	▸ For instable reagent absorbance and end point methods: When measuring a long series of samples, measure the reagent blank value not only at the beginning, but also during the sample series. If the blank reagent value drifts strongly, the reagent is not appropriate for error-free measurements and must be replaced by a new reagent.
	• The cuvette is not positioned correctly.	▸ Position the cuvette in the cuvette shaft so that the optical window points towards the direction of the light path. ▸ Photometry light path: from back to front

9.2 Error messages

You can exit device displays with error messages using the [OK] softkey.

System errors require an evaluation by the Technical Service. These errors are shown in English **(System error ...)**. Please contact Technical Service in these cases. Other error messages, for which you can carry out troubleshooting measures, are illustrated in the table below.

Problem	Cause	Solution
Self test failed.	• Cuvette shaft cover was open during self test. • The cuvette shaft was not empty during the self test.	▸ Repeat the self test with empty cuvette shaft and the cuvette shaft cover closed.
	• Device is faulty.	▸ Contact Eppendorf Service.
File export failed.	During data export: • USB stick improperly formatted or faulty. • USB stick removed from the device too early (during the export).	▸ Reformat or replace the USB stick. ▸ Reconnect the USB stick and repeat the export.
Failed to initialize printer.	• Printer not connected or switched off. • Printer not configured correctly.	▸ Connect the printer and switch it on. ▸ Reconfigure the printer. For a correct configuration of the printer settings refer to the installation description (see *Connecting the printer to the USB port on p. 18)*.
Blank measurement: An intensity on a pixel that influences the main, auxiliary or scan wavelength is too low.	• The absorbance of the blank solution used for the blank measurement is too high. • Incorrect or turbid blank solution. • For scans: Wavelength range is too large, because the sample is very strongly absorbed in part of the wavelength range.	▸ Check the blank solution and remeasure the blank if required. ▸ For scans: Match the wavelength range to the sample spectrum.
The entered name is not valid.	• Error when entering the name. Different causes are possible. For the precise cause please see the information in the help box.	▸ See information in the help box.
A method (or folder, dye, protein, nucleic acid, or unit) with this name already exists.	• The name under which the method was saved has already been used for a different method in the same folder. • The message also appears after editing names already given to a folder or to a nucleic acid (dye, protein, concentration unit) (under **General Method Parameter)**.	▸ Assign a different name.

Problem	Cause	Solution
The following parameter values are not defined in **General Method Parameter**:	• When opening a method with parameters which access **General Method Parameter**, the system determined that at least one parameter (dye, nucleic acid, protein, unit) does not exist there anymore, so probably has been deleted.	▸ Select a different parameter from the existing list. If necessary, program a new list entry in **General Method Parameter** in order to be able to use it when programming a method.
The value of the parameter marked with ***** is not defined in the Gen. Param. Please correct the parameter.	This error message appears when editing method parameters. • Parameter in **General Method Parameter** is not defined.	▸ Select a different parameter from the existing list. If necessary, program a new list entry in **General Method Parameter** in order to be able to use it when programming a method.
Invalid zoom interval.	During the Zoom process with free entry of limits ([Free]) softkey): • The zoom area is below the lower limit.	▸ Enter the values so that the interval does not fall below the range limits of 0.02 A and 10 nm.
The entered standard concentrations are not monotonically increasing resp. monotonically falling. Correct the standard concentrations.	• See the error text.	▸ Enter the standard concentrations so that the first standard receives the lowest concentration and the other standard concentrations form an increasing sequence.
At least two of the entered standard concentrations are identical. Correct the standard concentrations.	• See the error text.	▸ Enter the standard concentrations so that the first standard receives the lowest concentration and the other standard concentrations form an increasing sequence.
The measured values are not strictly monotonous!	• Error when measuring a standard series: The measured absorbance values of the standard series are not continuously increasing or decreasing.	▸ Repeat the standard measurements or delete the single, incorrectly measured standard result.
The ID cannot be set.	• Error when entering the sample ID. Different causes are possible. For the precise cause please see the information in the help box.	▸ See information in the help box.
The dilution cannot be set.	• Error when entering the dilution. Different causes are possible. For the precise cause please see the information in the help box.	▸ See information in the help box.

Problem	Cause	Solution
Calculation not possible because of division by zero. Absorbance result or Formula "b" parameter is zero.	• An absorbance result was divided by a "zero" value during the evaluation of a **Division** type method (**Dual wavelength** method group). This is not mathematically permissible.	▸ Check the reagents and samples used and repeat the measurement. ▸ Do not enter "zero" as a value for the Formula **b** parameter.
There is only one measurement left to be performed in this series of measurement. The maximum number of measurements within one series of measurements has been reached.	• The number of measurements in one measuring series is limited to 99.	▸ Start a new series of measurement after maximally 99 measurements.
Invalid zoom interval!	Error in the **process results** method step in the Zoom mode. Permissible zoom range for the wavelength scale: • Wavelength interval at least 10 nm • Entries for wavelengths only within the range programmed in the parameters for the method. Permissible zoom range for the absorbance scale: • Absorbance interval at least 0.02 A • Upper and lower limit for absorbance interval +3 A or –3 A	▸ Please observe the stated limits in the zoom procedure.
Device configuration was changed from … to … .	• A BioSpectrometer kinetics is not detected as the kinetic variant but as a BioSpectrometer basic. The kinetic methods are therefore not displayed.	▸ Switch the device off and back on again. If the error occurs again: Contact Technical Service.
The temperature control system is faulty. Please program the method without temperature control or cancel the method.	• The temperature control of the device is faulty.	▸ Contact Eppendorf Service. Until the temperature control has been repaired only use methods for which no temperature control has been programmed.

Problem	Cause	Solution
The ambient temperature is too high.	For kinetic methods with temperature control: • The ambient temperature measured by the device is above the specified range.	▸ Make sure that the ambient temperature is within the range specified for the operation of the device.
Linear regression could not be applied to all measurements.	• For kinetic methods, the time frame for evaluation with linear regression was changed in the **process results** method step, and the change should be extended to all measuring results. The required number of measuring points, however, was not available for at least one sample result.	▸ Only change the time frame for evaluation with linear regression in the **process results** method step for samples with sufficient measuring points.

9.3 Result flags

Warnings and error messages for results are displayed in the bottom right of the help box. The header bar of the Help box is highlighted yellow for warnings and red for error messages.

Warnings: Decide whether the result is useful for you while taking the displayed warning into consideration.

Error messages: No result is displayed; the reason is shown in the error message.

Problem	Cause	Solution
The standard curve is not monotone. Please select another Curve Fit.	• No usable result was returned during the evaluation of a standard curve using the "spline interpolation", "quadratic regression" or "cubic regression" **Curve Fit** procedures.	▸ Select a different **Curve Fit** procedure.
Some absorbance values for secondary wavelengths are too high or are not displayed.	• For at least one secondary wavelength, the absorbance exceeded the measuring range. • Secondary wavelengths are not needed for calculating the concentration result. They are used for different purposes. For example, **dsDNA** method: absorbance at 280 nm for the calculation of **ratios 260/280**. • Turbidity of the measuring solution • Measurements at the limits of the photometric measuring range.	▸ If the absorbance values of the secondary wavelengths are relevant: Dilute the sample or remove the turbidity via centrifugation and repeat the measurement.
The result is outside the range of the standard concentrations.	• For methods with evaluation via standard curves (nonlinear evaluation method): The sample result is up to 5 % outside of the standard concentration range.	▸ Accept the measurement result, or remeasure the sample under conditions under which the result is within the range of the standard concentrations (dilute sample or modify standard concentrations and remeasure).
The coefficient of determination is <0.8.	• For methods with evaluation of standard series via the regression procedure: The coefficient of determination for the regression evaluation indicates a significant deviation of the measuring points from the regression line. • Turbidity of the measuring solution. • Measurements at the limits of the photometric measuring range.	▸ Accept the result of the standard evaluation or remeasure the standards. ▸ Make sure the measuring solutions are clear.

Problem	Cause	Solution
The coefficient of determination for the regression evaluation of the standard series is < 0.8.	• For methods with evaluation of standard series via the regression procedure: If the regression evaluation for the standard series was nonlinear, but the standard evaluation was accepted by the user, a warning appears after samples have been measured.	▸ Use the sample results with the reservation mentioned or repeat the measurement of the standard series and samples.
Scan: Some of the measured absorbances are too high and are not displayed.	• For at least one scan wavelength, the absorbance exceeded the measuring range. • Turbidity of the measuring solution. • Measurements at the limits of the photometric measuring range.	▸ If the non-displayed areas of the scan are relevant: Dilute the sample or remove the dilution via centrifugation and repeat the measurement.
The measurement is not complete.	• Kinetic procedure: You have prematurely canceled the measurement using the [Stop] softkey. If at least 2 measuring points are available, a result is calculated and displayed.	▸ Accept the measuring result with the reduced measuring time or measure again with a longer measuring time.
The kinetics are nonlinear: The coefficient of determination is < 0.95.	• Kinetic procedure with "linear regression" measuring procedure: The coefficient of determination for the regression evaluation indicates a significant deviation of the measuring points from the regression line. • Turbidity of the measuring solution. • Measurements at the limits of the photometric measuring range. • Activity concentration of the enzyme too high.	▸ Accept measuring result or remeasure the sample. Before repeating the measurement, evaluate the reason for the non-linearity and act accordingly (e.g., clear the measuring solution by centrifugation or dilute the sample).
The kinetics are nonlinear for at least one standard: The coefficient of determination is <0.95.	• Kinetic procedure with "linear regression" measuring procedure and evaluation procedure via standards: The coefficient of determination for the regression evaluation of at least one standard measurement indicates a significant deviation of the measuring points from the regression line. • Turbidity of the measuring solution. • Measurements at the limits of the photometric measuring range. • Activity concentration of the enzyme too high.	▸ Accept measuring result or remeasure the standard. Before repeating the measurement, evaluate the reason for the non-linearity and act accordingly (e.g., clear the measuring solution via centrifugation or use the standard with low concentration).

Problem	Cause	Solution
During the kinetics measurement, the temperature was outside of the allowable range.	• Ambient temperature outside the specified range. • The temperature control is faulty.	▸ Measure the sample at ambient temperature within the specified range (15°C to 35°C). If the warning nevertheless appears, contact Eppendorf Service.
Absorbance at the measuring wavelength is too high.	• Turbidity of the measuring solution. • Optical surfaces of the cuvette are soiled. • Cuvette has been inserted into the cuvette shaft facing the wrong direction. • Too high absorbance of measuring solution.	▸ Measure again considering the possible causes.
The calculated result is negative.	• Measuring solution not prepared correctly. • The incorrect factor has been entered (wrong algebraic sign).	▸ Measure again considering the possible causes.
At least one of the results is negative.	• For methods with several results (e.g., **Dye labels**). • Measuring solution not prepared correctly. • The incorrect factor has been entered (wrong algebraic sign).	▸ Measure again considering the possible causes.
The result has more than 6 pre-decimal places.	• Very high sample concentration. • Concentration unit does not match the expected range of the sample concentrations.	▸ Dilute sample and measure again. ▸ Change the concentration unit (Parameter **Unit**) and measure again.
The result is more than 5 % outside of the standard concentration range.	• For methods with evaluation via standard curves (nonlinear evaluation method): The sample result is more than 5 % outside of the standard concentration range.	▸ Remeasure the sample under conditions under which the result is within the range of the standard concentrations (dilute sample, modify standard concentrations and remeasure).
• Calculation not possible because of division by zero. Absorbance result is zero. • Calculation error. Division by zero.	• The evaluation required dividing by an absorbance result with the value of "zero". This is not mathematically permissible. Examples: Calculation of a factor at one-point calibration; calculation of a 260/280 ratio with nucleic acid measurements.	▸ Check the reagents and samples used and repeat the measurement.

Problem	Cause	Solution
Calculation not possible because of division by zero. Absorbance result or parameter formula **b** is zero.	• An absorbance result was divided by a "zero" value during the evaluation of a **Division** type method (**Dual wavelength** method group). This is not mathematically permissible.	▸ Check the reagents and samples used and repeat the measurement. ▸ Do not enter "zero" as a value for the Formula **b** parameter.

10 Transport, storage and disposal

10.1 Transport

- Use the original packaging for transport.

	Air temperature	Relative humidity	Atmospheric pressure
General transport	-25 °C – 60 °C	10 % – 95 %	30 kPa – 106 kPa
Air freight	-40 °C – 55 °C	10 % – 95 %	30 kPa – 106 kPa

10.2 Storage

	Air temperature	Relative humidity	Atmospheric pressure
In transport packaging	-25 °C – 55 °C	25 % – 75 %	70 kPa – 106 kPa
Without transport packaging	-5 °C – 45 °C	25 % – 75 %	70 kPa – 106 kPa

10.3 Disposal

In case the product is to be disposed of, the relevant legal regulations are to be observed.

Information on the disposal of electrical and electronic devices in the European Community:

Within the European Community, the disposal of electrical devices is regulated by national regulations based on EU Directive 2012/19/EU pertaining to waste electrical and electronic equipment (WEEE).

According to these regulations, any devices supplied after August 13, 2005, in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. To document this, they have been marked with the following identification:

Because disposal regulations may differ from one country to another within the EU, please contact your supplier if necessary.

11 Technical data

11.1 Power supply

Power supply	100 V to 240 V ±10 %, 50 Hz to 60 Hz
Overvoltage category	II
Degree of pollution	2
Power consumption	Maximum power consumption according to name plate: 50 W Approx. 30 W during operation Approx. 5 W with the display dimmed and temperature control switched off
Permitted mains interruption	Approx. 10 ms at 90 V Approx. 20 ms at 230 V
Protection class	I
Fuses	T 2.5 A/250 V, 5 mm × 20 mm (2 pcs.)

11.2 Ambient conditions

Operation	Ambient temperature: 15°C to 35°C Rel. humidity: 25% to 70% Air pressure: 86 kPa to 106 kPa
Air pressure	Use up to an altitude of 2000 m above MSL

Do not expose to direct sunlight.

11.3 Weight/dimensions

Weight	5.3 kg
Dimensions	Width: 295 mm Depth: 400 mm Height:150 mm
Space required	Width: 500 mm (with thermal printer: 750 mm) Depth: 500 mm

11.4 Photometric properties

Measuring principle	Single beam absorption spectrophotometer with reference beam
Light source	Xenon flash lamp
Monochromator	Holographic aberration-corrected concave grating
Beam receiver	CMOS photodiode array
Wavelengths	200 nm to 830 nm
Wavelength selection	Method-dependent, freely selectable
Spectral bandwidth	≤ 4 nm
Smallest step size	1 nm
Systematic wavelength error	±1 nm
Random wavelength error	≤ 0.5 nm
Photometric measuring range	0 A to 3.0 A at 260 nm
Reading accuracy	ΔA = 0.001
Random photometric error	≤ 0.002 at A = 0 ≤ 0.005 (0.5 %) at A = 1
Systematic photometric error	±1 % at A = 1
Stray light component	< 0.05 %

11.5 Incubation

Adjustable temperature range	20°C to 42°C
Smallest step size	0.1°C
Maximum temperature uncertainty (in relation to the temperature in the sample)	(see Tab. on p. 30)
Systematic temperature error	±0.2 °C at 25 °C to 37 °C
Random temperature error	±0.15 °C at 25 °C to 37 °C

11.6 Further technical parameters

Cuvette material	For measurements in the UV: Quartz glass or UV transparent plastic (Eppendorf UVette, 220 nm to 1600 nm) For measurements in the visible range: Glass or plastic material
Cuvette shaft	12.5 mm × 12.5 mm, tempered
Overall cuvette height	Min. 36 mm
Height of the light beam in the cuvette	8.5 mm
Key pad	22 foil keys 6 foil keys as softkeys
Result output	Absorbance, concentration, scan (absorbance wavelength spectrum) Additional, method-dependent data (ratio, FOI, background absorbances)
Display	VGA TFT display 5.7”
Operator guidance language	English, French, Spanish, Italian, German
Interfaces	USB master: for USB stick and thermal printer DPU-S445 USB slave for connecting to a PC Serial interface RS 232: for thermal printer DPU-414 Ethernet interface RJ45: For connecting to a network Connected devices must meet the safety requirements specified in IEC 60950-1.

11.7 Application parameters

Methods	Preprogrammed and freely programmable methods for all measuring and evaluation procedures: • Absorbance measurements with one or more wavelengths, scans • Nucleic acids and proteins, OD600, dye methods (parallel measurement of biomolecule and dye marking) • Methods with evaluation via factor, standard and standard series • Dual wavelength procedure with subtraction and division evaluation • Kinetic method: End point, two-point, linear regression
Method-dependent evaluation	Absorbance, concentration via factor and standard. Concentration via standard series: • Linear regression • Nonlinear regression (2nd and 3rd degree polynoms) • Spline evaluation • Linear interpolation (point-to-point evaluation) Absorbance calculations via subtraction and division Additional data for nucleic acids: Ratios 260/280 and 260/230; molar concentration, total yield Additional data for dye methods: FOI (frequency of incorporation, labeling density) Scans: zoom, peak evaluation Kinetics: Subsequent modification of the time window for the regression evaluation
Method memory	>100 method programs
Measured value memory and calibration memory	Memory for >1 000 results with all data of the results evaluation and standard evaluation, sample number, sample name, date and used parameter set of the method program. (The number of saved results depends on the number of saved methods.)

12 Evaluation procedure

This chapter describes the evaluation procedures available in the method programs as well as the calculation of a dilution using the device software.

When comparing the measuring results to the results of other photometers/ spectrophotometers, note that the values may be dependent on the bandwidth of the devices. In the following cases the differences may be significant:

- The absorbance spectrum shows a narrow peak in the measurement wavelength.
- The measurement is carried out not at the maximum but at the edge of a peak.

Therefore, check the accuracy of the methods by measuring standards.

12.1 Absorbance values

Absorbance values are displayed as A_{XXX} (XXX represents the wavelength). These displays always match the directly measured values, i.e., without corrections, which are incorporated in the final evaluation, e.g., corrections for optical path lengths of the cuvette, or background corrections.

12.1.1 Blank

All absorbance values are always related to the last measured blank (blank). Therefore, a blank measurement is compulsory at the start of every series of measurements and can be completed at any time during a series of measurements. Ideally, the blank measurement should be able to compensate for any influences on the absorbance value of the measuring solution. The blank should therefore be measured with the same buffer that was used for the sample measurement and the same cuvette that was used to measure the sample value – unless the cuvettes used for the blank and sample measurements are optically aligned and thus have the same absorbance value at the measuring wavelength.

12.1.2 Background correction

Main application: Partial correction of distortions of the absorbance for nucleic acid measurements due to turbidity in the measuring solution. For example, the absorbance at 320 nm, which should be approx. 0 A with pure nucleic acids, is subtracted from the absorbance at 260 nm, (the measuring wavelength for nucleic acids).

$$A_{XXX,corrBkgr} = A_{XXX} - A_{Bkgr}$$

$A_{XXX,\ corrBkgr}$ = calculated corrected absorbance at a wavelength of XXX nm.

A_{XXX} = measured absorbance at a wavelength of XXX nm.

A_{Bkgr} = measured absorbance at the background wavelength.

12.1.3 Cuvette correction

All absorbance values which are used for result calculation are standardized to the cuvette layer thickness of 10 mm. If a cuvette with a different path length is used, this path length must be defined in the **cuvette** parameter. In this case, the measured absorbances are corrected to match measuring results with a cuvette layer thickness of 10 mm before converting them to sample results.

This correction is applied to:
- Methods with evaluation by factor.
- Methods of the ***Absorbance*** group, for which only absorbance values are output

The correction is not applied to:
- Methods with evaluation by standards, as we presume that standards and samples are measured in cuvettes of the same layer thickness.
- Calculations with division: **Division** method (**Dual wavelength** method group) and calculation of ratios such as A_{260}/A_{280} (for nucleic acid measurements).

$$A_{XXX,corrCuv} = A_{XXX} \times \frac{10}{Cuv}$$

$A_{XXX,\ corrCuv}$ = calculated corrected absorbance at a wavelength of XXX nm.

A_{XXX} = measured absorbance at a wavelength of XXX nm.

Cuv = path length of the cuvette.

12.2 Evaluation with factor or standard

$$C = A \times F$$

C = calculated concentration.

A = absorbance.

F = factor.

The factor is programmed in the parameter list and can be modified. It always relates to an optical path length of the cuvette of 10 mm. If you change the **Cuvette** parameter the device will take the modification into account when calculating the results. Therefore you do not need to change the factor for the evaluation.

If, on the other hand, you modify the concentration unit, you have to ensure that the factor is adjusted for the selected unit.

The factor is either entered directly as a parameter during the "Factor" evaluation procedure or calculated during the "Standard" evaluation procedure (evaluation with a standard concentration):

$$F = \frac{C_S}{A_S}$$

F = calculated factor

C_S = concentration of the standard (enter as parameter).

A_S = measured absorbance of the standard.

If multiple measurement (2 or 3 replicates) has been programmed for the standard, the average value is calculated from the measured absorbance values and inserted as A_S.

12.3 Evaluation with standard curve/line

If evaluations are made with more than one standard, the following evaluation procedures for the standard curve/line can be selected with the [Curve fit] in the **measure standards/new** method step:

Evaluation procedure	Description	Minimum required number of standard points
Linear interpolation	Linear point-to-point connection in the absorbance concentration graph of the standard evaluation.	2 standards minimum.
Linear regression	Polynome regression for first degree polynomial.	3 standards minimum.
Quadratical regression	Polynome regression for second degree polynomial.	4 standards minimum.
Cubical regression	Polynome regression for third degree polynomial.	5 standards minimum.
Spline interpolation	Interpolation via natural cubic splines.	3 standards minimum.

For the regression procedure, one can select that the regression line (regression curve) goes through the zero point.

- Use the "linear regression" procedure for calibration lines.
- With curvilinear gradients, test which evaluation procedure (quadratic regression, cubic regression, spline interpolation) produces the function that is most suitable to the standard evaluation. Spline interpolation connects the measuring points by cubic polynomials, whereas the regression methods position a quadratic or cubic function between the measuring points in such a way, that the smallest possible deviation from the function results for the measuring points.
- Aside from the calculated regression equation, the regression method also displays the coefficient of determination as a measure for the scattering of the measuring points around the calculated function. At a value of < 0.8 for the coefficient of determination the result is issued with a warning.
- If the first standard hat a concentration of "0", select the setting in which the regression line (regression curve) goes through the zero point.
- If none of the procedures recommended for curvilinear gradients produce satisfactory results, select the "linear interpolation" procedure.

12.4 Dilution

In the **measure samples** method step. entered dilutions are considered in the result calculation:

$$C_{Dil,korr} = C \times \frac{V_P + V_{Dil}}{V_P}$$

$C_{Dil,\ corr}$ = result converted using the dilution factor

V_S = volume of the sample in the measuring solution

V_{Dil} = volume of the diluent in the measuring solution

12.5 Special evaluation procedures for nucleic acids and protein UV

This section covers the evaluation of nucleic acids or proteins in the **Nucleic acids** and **Proteins direct UV** method groups, as well as the corresponding biomolecular components in the **Dye labels** method group.

12.5.1 Correction A_{260} and correction A_{280}

Application: correction of the influence of dye absorbance on the nucleic acid or protein absorbance at 260 and 280 nm for the methods of the **Dye labels** group.

The application of the evaluation procedure can be activated in the parameters **Correct A260** or **Correct A280**.

$$A_{XXX,corr} = A_{XXX} - CF \times A_{YYY}$$

$A_{XXX,\ corr}$ = calculated corrected absorbance for a wavelength of 260 nm or 280 nm

A_{XXX} = measured absorbance for a wavelength of 260 nm or 280 nm

CF = correction factor for a wavelength of 260 nm or 280 nm (the correction factors for 260 nm and 280 nm are both dye-specific and are programmed in **General Method Parameter: Dyes** in the **Functions** area).

A_{YYY} = measured absorbance at the dye wavelength.

The absorbance values displayed in the results are the directly measured, not the corrected absorbance values.

12.5.2 Ratios A260/A280 and A260/A230.

Application: Information on the purity of the measured nucleic acid. Application of the evaluation procedure can be activated in the **A260/280** or **A260/A230** parameters.

"Ratio" refers to the quotients of the measured absorbances at the listed wavelengths.

Literature values for ratio values with pure nucleic acids:

A260/A280
- DNA: 1.8 to 1.9
- RNA: 1.9 to 2.0
  (Current Protocols in Molecular Biology, 1994)

A260/A230

For the ratios A260/A230, different information can be found in the literature for pure nucleic acids:
- DNA: 2.3 to 2.5
  (The Nucleic Acids, 1955)
- DNA: 1.9
  (Current Protocols in Molecular Biology, 1994)

The values are highly dependent on the pH value. Therefore, nucleic acids should not be measured in water, but in a buffer with a pH of 7 to 7.2 (e.g., TE buffer).

12.5.3 Conversion to molar concentrations and nucleic acid quantities

The conversion only can be applied to nucleic acids and dye methods with nucleic acids as biomolecule components. It is realized in the **process results/More calculations** method step.

12.5.3.1 Calculation of amount

Application: calculating the amount (mass) of nucleic acid in the total sample volume.

$$M = C \times V_{P,gesamt}$$

M = calculated total amount (mass) of nucleic acid in the sample tube. Unit: µg.

C = nucleic acid concentration calculated from the measurement. Unit: µg/mL or ng/µL.

$V_{S,\ total}$ = total volume of the sample in the sample tube. Enter this value in **More calculations**. Unit: µL.

12.5.3.2 Calculation of the molar concentration

Application: calculation of the molar concentration of the nucleic acid from the mass concentration and relative molar mass. The molar mass is either entered directly or calculated by the device from the entered number of bases or base pairs per nucleic acid molecule.

$$C_{Mol} = \frac{C \times 10^3}{MM}$$

C_{Mol} = calculated molar concentration of the nucleic acid. Unit: pmol/mL.

C = nucleic acid concentration calculated from the measurement. Unit: µg/mL or ng/µL.

MM = relative molar mass. Unit: kDa

If the number of bases or base pairs per nucleic acid molecule are entered in **More calculations** instead of the relative molar mass, the MM is calculated from the number of the bases or base pairs:

For **dsDNA:**

$$MM = bp \times 2 \times 330 \times 10^{-3}$$

For **ssDNA, RNA, Oligo:**

$$MM = b \times 330 \times 10^{-3}$$

MM = calculated relative molar mass; unit: kDa

bp = entered number of base pairs per molecule

b = entered number of bases per molecule

- For **dsDNA** the calculation of the molar concentration is based on the assumption of a double-stranded nucleic acid. For the **ssDNA**, **RNA** and **Oligo** methods, a single-stranded nucleic acid is assumed.
- For methods which have been reprogrammed via **<New Method>** in the ***Routine*** main group, **Nucleic acids** method group, always double-stranded nucleic acids are assumed for calculating the molar concentration.

12.5.4 Calculating the factor for protein in "General Method Parameter"

This section only covers the calculation of the protein components in the **Dye labels** and **Proteins direct UV** method groups. For these method groups, the protein component is selected in the parameters (see *Method parameters on p. 38)*. The protein component is assigned a factor that will be entered in the **General Method Parameter/Proteins** function for each protein. Alternatively, $A_{0.1\%}$ or the absorbance coefficient plus the molar mass of the protein can be entered instead of the factor. In this case, the factor is calculated as follows:

$$F_P = \frac{1}{A_{0.1\%}}$$

F = factor for the protein; unit: g/L.

$A_{0.1\%}$ = absorbance of the protein at a concentration of 0.1 % (1 g/L).

When entering the molar absorbance coefficient and the relative molar mass of the protein $A_{0.1\%}$ can be calculated on this basis:

$$A_{0.1\%} = \frac{\varepsilon_P}{MM_P}$$

ε_P = molar absorbance coefficient of the protein; unit: $cm^{-1}M^{-1}$.

MM_P = relative molar mass of the protein; unit: Da (entry in **General Method Parameter** in kDa).

12.6 Special evaluation procedures for the dye methods

12.6.1 Calculating the factor for the dye from the absorbance coefficient

For the dye methods the concentration of the dye is calculated using a factor from the measured absorbance (see *Evaluation with factor or standard on p. 105)*. The factor is entered for each dye in the **General Method Parameter/Dyes** function. Alternatively, you can enter the absorbance coefficient. In this case, the factor is calculated as follows:

$$F_{Dye} = \frac{10^6}{\varepsilon_{Dye}}$$

F = factor for the dye; unit: pmol/µL.

ε = absorbance coefficient for the dye, unit: $cm^{-1}M^{-1}$

12.6.2 Calculation of the FOI

As a value for the ratio of dye molecules to the number of nucleotides in the nucleic acid the frequency of incorporation (FOI) is calculated and displayed for the dye methods. The calculation can be selected for two different result units:

MOLECULE dye/kb unit

$$FOI = \frac{A_{YYY}}{\varepsilon_{Dye}} \times \frac{10^6 \times MM_{nt}}{A_{XXX} \times F_{NA}}$$

pmole/µg DNA (or RNA) unit

$$FOI = \frac{A_{YYY}}{\varepsilon_{Dye}} \times \frac{10^9}{A_{XXX} \times F_{NA}}$$

A_{YYY} = absorbance of the dye.

A_{XXX} = absorbance of the nucleic acid.

MM_{nt} = average molar mass of the nucleotides: 330 g/mol.

F_{NA} = factor for calculating the nucleic acid

ε_{dye} = absorbance coefficient for the dye, unit: $cm^{-1}M^{-1}$

12.6.3 Conversion to amounts of dye

The quantity (dimensions) of dye in the entire sample volume is calculated in the **process results/More calculations** method step.

$$M = C \times V_{P,total}$$

M = calculated total amount (mass) of dye in the sample tube. Unit: pmol.

C = dye concentration calculated from the measurement. Unit: pmol/µL.

$V_{S,\ total}$ = total volume of the sample in the sample tube; entered by the user under **More calculations**. Unit: µL.

12.7 Dual wavelength

For methods of the **Dual Wavelength** group absorbances that were measured at two wavelengths can be calculated with each other before the calculated absorbance is evaluated further with the factor or standard.

To determine the calculated absorbance a division or subtraction evaluation can be defined in the parameters:

$$A_{calc} = \frac{a \times A_1}{b \times A_2} \times c + d$$

$$A_{calc} = \left[\left(a \times A_1\right) - \left(b \times A_2\right)\right] \times c + d$$

A_1, A_2 = measured absorbance.

a, b, c, d = factors that are entered in the parameters. Negative figures may also be entered.

12.8 Kinetics

An absorbance value **A** or an absorbance difference standardized to a minute **ΔA/min** is determined using the measurement procedures available for selection. This determined absorbance value is entered in the concentration calculation using a factor or (only for **Advanced kinetics**) with an end-point standard.

12.8.1 Measurement procedures

Endpoint

At the end of the waiting period **(incubation period, Delay** parameter), an absorbance value is measured and used for the concentration calculation.

Two point

After the delay the measuring point is taken and after the measuring time has expired a second one is taken. ΔA/min is calculated from the absorbance and the time difference.

$$\frac{\Delta A}{\min} = \frac{A_2 - A_1}{t_2 - t_1}$$

A_1, t_1 = absorbance and time for the first measuring point.

A_2, t_2 = absorbance and time for the second measuring point.

Linear regression

After the delay has expired measuring points are recorded at fixed time intervals from the start to the end of the measuring time. A linear regression is carried out via the measuring points in the absorbance/time diagram. Result: absorbance value in ΔA/min.

12.8.2 Reagent blank value

For the methods of the **Advanced kinetics** group, the measurement of a reagent blank can be preprogrammed in the parameters. Primary application: compensation of a reagent drift in the "Linear regression" measurement procedure. Instead of a sample, the reagent blank contains the reagent and demineralized water, and is measured according to the same measurement procedure as the sample. The measurement is made at the start of the sample series; the absorbance result is then subtracted from the sample absorbance result before the evaluation:

$$A_{RB,corr} = A - A_{RB}$$

$A_{RB,\ corr}$ = corrected absorbance result (in A or ΔA/min) of the sample.

A = measured absorbance result (in A or ΔA/min) of the sample.

A_{RB} = absorbance result (in A or ΔA/min) of the reagent blank.

If the time frame for the kinetic evaluation with linear regression is reduced in the **process results** method step, the reduced measuring window is automatically used for calculating the reagent blank which was incorporated in the calculation of this sample result. That means that the reagent blank is recalculated (only) for the calculation of this sample result. For the calculation of the other sample results the reagent blank result that was calculated on the basis of the originally used time window is used.

When the kinetic method is evaluated with standards the measurement of a reagent blank is not available. Here, the reagent blank can alternatively be defined as the first standard ("zero" concentration).

13 Ordering information

Order no. (International)	Order no. (North America)	Description
		Eppendorf BioSpectrometer basic
6135 000.009	–	230 V/50 – 60 Hz, mains/power plug Europe, more types of mains/power connection available
6135 000.017	6135000017	120 V/50 – 60 Hz, mains/power plug North America
		Eppendorf BioSpectrometer kinetic
6136 000.002	–	230 V/50-60 Hz, 230 V/50 – 60 Hz, mains/power plug Europe, more types of mains/power connection available
6136 000.010	6136000010	120 V/50-60 Hz, mains/power plug North America
		BioSpectrometer reference filter set
6135 928.001	6135928001	Filter set for checking photometric precision and wavelength accuracy (according to NIST)
		Eppendorf µCuvette G1.0
6138 000.018	6138000018	Eppendorf microvolume measuring cell for Eppendorf BioPhotometer and BioSpectrometer
		Thermal Printer DPU-S445
		including power supply and printer cable
6135 011.000		230 V, EU
6135 010.004	6135010004	115 V/110V, USA, JP
6135 012.007		230 V, UK
		Thermo paper
0013 021.566	952010409	5 rolls
		Eppendorf UVette 220 nm – 1 600 nm
		Original Eppendorf plastic cuvette, PCR clean, Protein-free
0030 106.300	952010051	50 - 2 000 µL, 80 pieces, individually packaged
		Eppendorf UVette routine pack 220 nm – 1 600 nm
		Eppendorf Quality
0030 106.318	952010069	50 - 2 000 µL, 200 pieces, reclosable box
		Eppendorf macro Vis Cuvettes
0030 079.345	0030079345	10 × 100 pieces
		Eppendorf semi-micro Vis Cuvettes
0030 079.353	0030079353	10 × 100 pieces
		Cuvette stand
4308 078.006	940001102	for 16 cuvettes

EG-Konformitätserklärung
EC Conformity Declaration

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produktbezeichnung, Product name:

Eppendorf BioSpectrometer® kinetic

Produkttyp, Product type:

Photometer

Einschlägige EG-Richtlinien/Normen, Relevant EC directives/standards:

2006/95/EG; EN 61010-1

2004/108/EG; EN 55011, EN 61326-1

2011/65/EU

Vorstand, Board of Management:

Projektmanagement, Project Management:

07.08.2013

Hamburg, Date:

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