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# Eppendorf BioPhotometer® D30

Operating manual

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### **Notice**

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## 1 Operating instructions






### 1.1 Using this manual

- ▶ Read this operating manual thoroughly 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. It must always be kept 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 website at [www.eppendorf.com/manuals](http://www.eppendorf.com/manuals).

### 1.2 Danger symbols and danger levels

#### 1.2.1 Danger symbols


The safety instructions in this manual have the following danger symbols and danger levels:

	<b>Electric shock</b>		<b>Explosive substances</b>
	<b>Toxic substances</b>		<b>Hazard point</b>
	<b>Material damage</b>		

#### 1.2.2 Danger levels

<b>DANGER</b>	<i>Will lead to severe injuries or death.</i>
<b>WARNING</b>	<i>May lead to severe injuries or death.</i>
<b>CAUTION</b>	<i>May lead to light to moderate injuries.</i>
<b>NOTICE</b>	<i>May lead to material damage.</i>

### 1.3 Symbols used

Depiction	Meaning
1. 2.	Actions in the specified order
▶	Actions without a specified order
•	List
	Additional information

**Operating instructions**

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**1.4 Abbreviations used****A**

Absorbance

**DNA**

Deoxyribonucleic acid (DNA)

**dsDNA**

Double-stranded DNA

**M**

mol/L (*molar*)

**OD600**

Optical density at a wavelength of 600 nm

**RNA**

Ribonucleic acid

**ssDNA**

Single-stranded DNA

**T**

Transmission: The transmission (T), which is the light transmittance of the cuvette, is calculated using the ratio of I (light exiting the cuvette) and  $I_0$  (light entering the cuvette):  $T = I/I_0$

**UV**

Ultraviolet radiation

**Vis**

Visible light

**CV**

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

## 2 Safety

### 2.1 Intended use

The BioPhotometer D30 is to be used in molecular biology, biochemistry and cell biology research laboratories. The BioPhotometer D30 exclusively is determined for use in the interior of buildings. All country-specific safety requirements for operating electrical equipment in the laboratory must be observed.

The BioPhotometer D30 is used for photometric concentration determination of biomolecules in liquids as well as for turbidity measurements of microbiological cultures in routine laboratories.

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 due to the ingress of liquid.**

- ▶ Switch off the device and disconnect it from the mains/power line before starting cleaning or disinfection.
- ▶ 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 once it is completely dry, both inside and outside.



**DANGER! Risk of explosion.**

- ▶ Do not operate the device in areas where work is carried out with explosive substances.
- ▶ Do not use this device to process any explosive or highly reactive substances.
- ▶ Do not use this device to process any substances which could create an explosive atmosphere.



**WARNING! Electric shock due to damage to device or mains/power cord.**

- ▶ Only switch on the device if the device and the mains/power cord are undamaged.
- ▶ Only operate devices which have been installed or repaired properly.
- ▶ In case of danger, disconnect the device from the mains/power supply voltage. Disconnect the mains/power plug from the device or the earth/grounded socket. Use the isolating device intended for this purpose (e.g., the emergency 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 your personal protective equipment.
- ▶ Consult the "Laboratory Biosafety Manual" (source: World Health Organization, Laboratory Biosafety Manual, in its respectively current valid version).

**WARNING! Risk 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, clean it immediately using a mild cleaning agent.



**NOTICE! Damage to the device due to gassing with aggressive chemicals.**

- ▶ Do not use gassing to disinfect the device.



**NOTICE! Corrosion due to aggressive cleaning agents and disinfectants.**

- ▶ Do not use any corrosive cleaning agents, aggressive solvents or abrasive polishes.
- ▶ Do not incubate the accessories in aggressive cleaning agents or disinfectants for longer periods.



**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 for at least 3 h. Only then connect the device to the mains/ power line.



**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 due to overheating.**

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



**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 any doubts, contact the manufacturer of this product.

**Safety**

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**NOTICE! Damage as a result of improper packing.**

Eppendorf AG is not liable for any damage caused by improper packing.

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

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


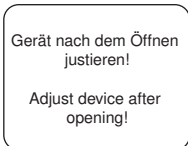
- ▶ Only clean the cuvette shaft using a moist cotton swab .
- ▶ Do not allow any liquid to enter the cuvette shaft.
- ▶ Do not reach with your fingers into the cuvette shaft.

## 2.4 Information on product liability

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

- 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 that are not recommended by Eppendorf.
- The device is maintained or repaired by persons not authorized by Eppendorf AG.
- 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
	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 Product overview**

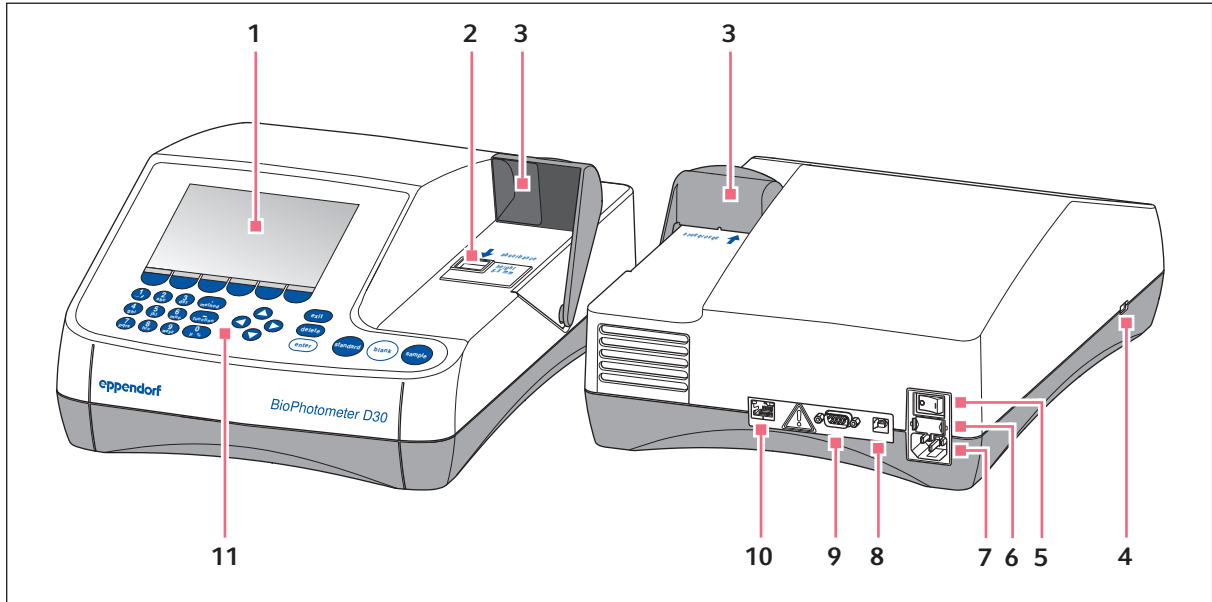


Fig. 3-1: BioPhotometer D30: Front and rear view

- 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 port
- 11 Operating controls

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

**3.2 Delivery package**

Quantity	Description
1	BioPhotometer D30
1	Power cord
4	4 UVettes Original Eppendorf plastic cuvette, individually packaged, PCR clean, protein-free
1	Operating manual, in multiple languages

**Product description**

Eppendorf BioPhotometer® D30  
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### 3.3 Features

The BioPhotometer D30 is a UV VIS photometer for measuring liquids in cuvettes. As the measuring data are collected at fixed wavelengths, the device especially is suited for routine applications in the biomolecular, biotechnological, biochemical and cytological areas in research and development.

#### 3.3.1 Methods

Preprogrammed methods and method templates

- Concentration determination of nucleic acids and proteins
- Determination of the bacterial density by turbidity measurements: Method **OD 600**
- Method templates for different measurement and evaluation procedures:
  - Quick absorbance measurements
  - Evaluations with factor, standard and standard curve
- Individual methods can be created on the basis of the preprogrammed methods and templates.
- Methods for quick and easy absorbance and transmission measurements without any further evaluation: **Absorbance** method group.

#### 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. If required, a help box in the display will provide you with hints. The 3 measuring keys (**standard, blank, sample**) enable you to start the measurement quickly and directly.

#### 3.3.3 Result output

The BioPhotometer D30 outputs the results via the device display or via a printer available from Eppendorf. With a USB port, 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

Directly after switching on, the device checks the functioning of the photometer unit by itself. Access the **Device calibration** function for a more comprehensive test (see *Device self test on p. 65*).

## 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. 13*).
- ▶ Check all parts for any transport damage.

### 4.2 Selecting the location

Select the location for the BioPhotometer D30 according to the following criteria:

- 2 grounded sockets for the BioPhotometer D30 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 BioPhotometer D30 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. 55*)

### Prerequisites

Ethernet cable (RJ45)

1. Connect the Ethernet cable to the network connection socket.
2. Connect the Ethernet cable to the Ethernet port **10** (see *Product overview on p. 13*).



### 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. 55*).

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 *Product overview on p. 13*).
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 *Product overview on p. 13*).

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 *Product overview on p. 13*).



- 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. 44*) method step.

**Installation**

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## 5 Operation





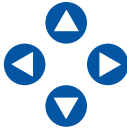






### 5.1 Operating controls



Fig. 5-1: Control panel of the BioPhotometer D30

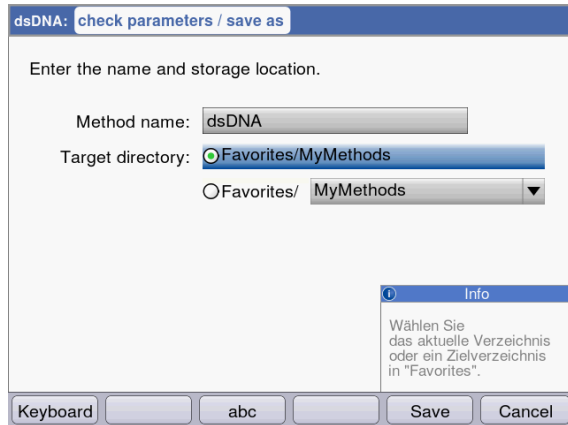
## Operation

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Key:	Function
	<p>Keypad: Enter digits and text.</p> <p>Keys <b>1</b> to <b>9</b> as well as <b>0</b>: 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.</p>
	<p>Outside of entry fields: Call up method selection.</p>
	<p>Outside of entry fields: Call up function selection.</p>
	<p>Softkey: Select functions.</p> <p>The key assignment changes along with the software dialog. The current function is displayed directly above the key on the display.</p>
	<p>Move the cursor to the left, right, up, down.</p> <ul style="list-style-type: none"> <li>• Navigation between input fields.</li> <li>• ◀ and ▶ keys inside an entry field: Navigate within the character string.</li> <li>• ▲ and ▼ keys in a result display: Navigate between the sample results of the series of measurement.</li> <li>• ◀ 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.</li> </ul>
  	<p>Exit the current selection for the next higher level.</p> <p>Delete entry. Within a sequence of signs, the sign on the left of the cursor is deleted</p> <ul style="list-style-type: none"> <li>• Call up selected method or function.</li> <li>• Open the selection list.</li> <li>• Confirm entry or selection.</li> </ul>
  	<p>Start standard measurement.</p> <p>Start blank measurement.</p> <p>Start sample measurement.</p>

### 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.

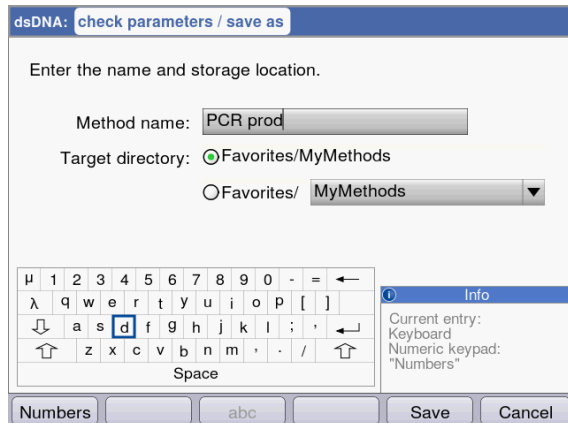


Entry via keyboard:

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.

## Operation

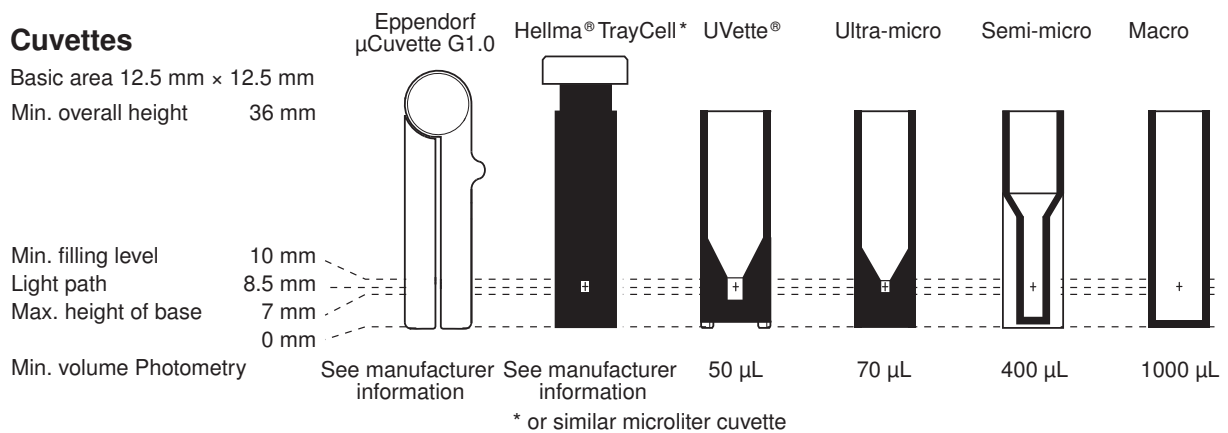
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### 5.2 Inserting the cuvette

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 cuvette temperature and temperature control of the cuvette are above the temperature of the dew point that applies for the ambient conditions (humidity and temperature).



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

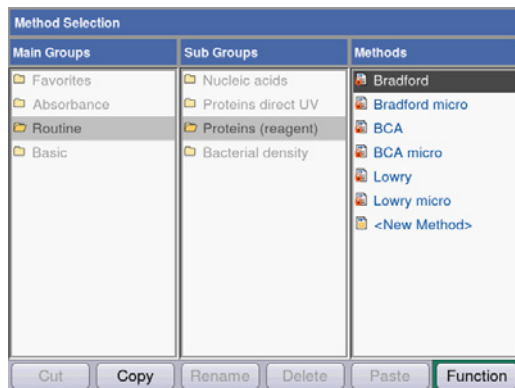
1. Switch on the device and, if required, the printer.  
The device performs a self test (taking approx. 1 minute) and displays the method selection.
2. Make ready the cuvettes for the measurements (see *Inserting the cuvette on p. 22*).
3. Prepare the measuring solutions for measuring the blank values, if required, also the standards and the samples.
4. Open the cover of the cuvette shaft. The cover can remain open during the measurements.



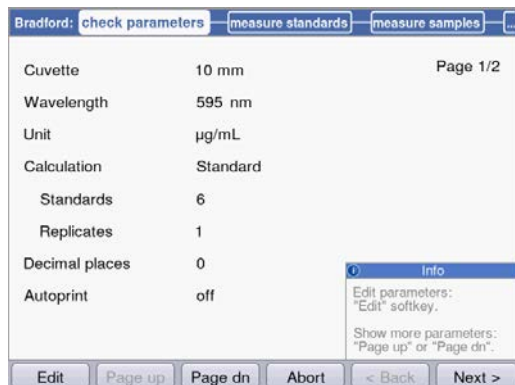
Measuring solutions for standards and samples with absorbance values lower than 0.05 A should not be used. The detection limit of the device may be significantly lower, nevertheless, the impact of disturbances from the measuring solutions (e.g. particles, bubbles, turbidity) on the reliability of the 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](http://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. 29*).



**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.

## Operation

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### 5.3.2.2 Checking parameters

Parameter	Value
Cuvette	10 mm
Wavelength	595 nm
Unit	µg/mL
Calculation	Standard
Standards	6
Replicates	1
Decimal places	0
Autoprint	off

Page 1/2

Info  
Edit parameters:  
"Edit" softkey.  
Show more parameters:  
"Page up" or "Page dn".

Edit Page up Page dn Abort < Back Next >

- ▶ 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.

Standard	Conc. µg/mL	Abs. A <sub>595</sub>
Standard 1	100	-
Standard 2	250	-
Standard 3	500	-
Standard 4	750	-

Linear regression:  
not calculated

Info  
Measure blank:  
"blank" key.

Last Cal Curve Fit Graph Abort < Back Next >

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] or [Table] softkey to change the result view.

Standard	Conc. µg/mL	Abs. A <sub>595</sub>
Standard 3	500	0.709
Standard 4	750	0.927
Standard 5	1000	1.047
Standard 6	1500	1.288

Quadratical regression:  
Conc. =  $924.41 \cdot A^2 - 134.52 \cdot A + 123.14$

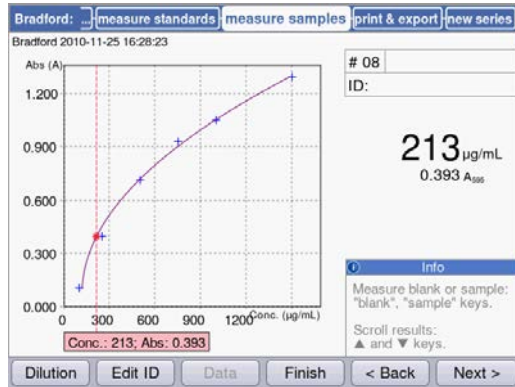
Coefficient of determination:  
 $R^2 = 0.9970$

Info  
Save evaluation and go to sample meas.:  
"Next >" softkey. Scroll standards/replicates ▲ and ▼ keys.

Last Cal Curve Fit Graph Abort < Back Next >

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

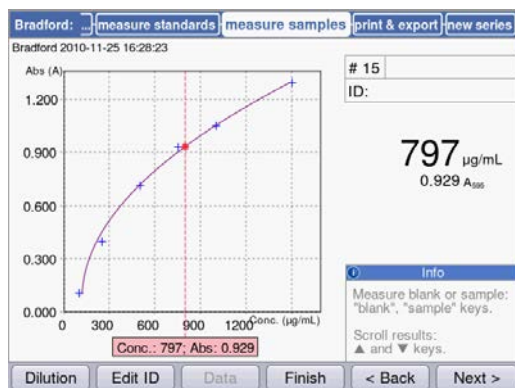
### 5.3.2.4 Measuring samples



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

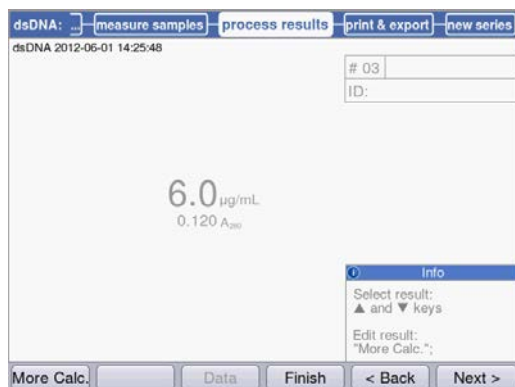
Blank results will remain saved for the duration of one series of measurements. However, a new blank measurement is always possible. (The adjacent figure shows a measuring procedure with evaluation via the standard curve and, in addition to the sample result, the graph of the standard evaluation.)

### 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

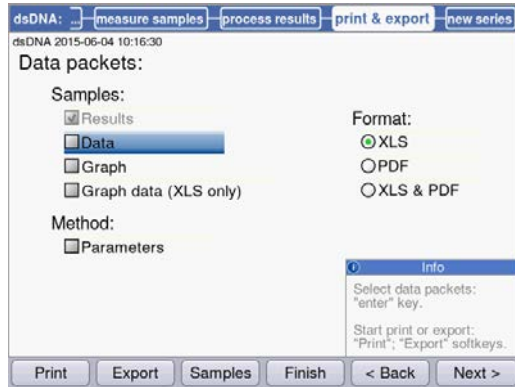


For the methods of the **Nucleic acids** method group, you can postprocess the results in the **process results** method step.

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

**More Calc.** softkey: Convert the concentration results to molar concentrations or to nucleic acid quantities (unit mass or mol).

### 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 will only be incorporated for the following result calculation (see *Absorbance values on p. 81*).
- The measuring result is typically displayed 2 to 3 seconds after a measurement has been started. If (for high absorbance values) only a small amount of light reaches the receiver, the measuring time can automatically be extended up to 9 seconds in order to increase the precision of the measurement.
- 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. 78*), 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  $\mu$ Cuvette 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.

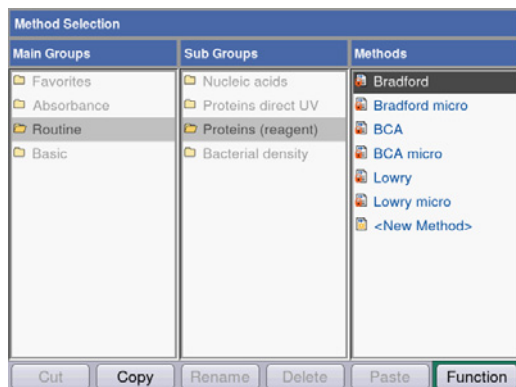
**Operation**

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## 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 up 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

<b>Absorbance</b>	Methods for quick and simple absorbance and transmission measurements without any further evaluations.
<b>Routine</b>	Frequently used molecular biology methods. The methods are preprogrammed. However, the parameters can be modified if saved under a new name.
<b>Basic</b>	Methods for the evaluation of absorbance measurements with factor, standard or standard curve/line.
<b>Favorites</b>	In <b>Favorites</b> , you can set up your own folders using <b>&lt;New folder&gt;</b> , 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.

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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 $\lambda$

- Absorbance measurement on a wavelength.
- No subsequent evaluation.
- Determination of the transmission of a sample is 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 a 1 mm light path (with microliter cuvettes such as Eppendorf  $\mu$ Cuvette G1.0 or Hellma® TrayCell).
- Partial turbidity correction via the **Background** parameter is preset.
- Additional information on the purity of the measured nucleic acid: ratio A260/A280, ratio A260/A230, restricted absorbance wavelength spectrum of nucleic acid (with 3 nm distances), absorbance of the background wavelength (preset: 320 nm; the absorbance of the pure nucleic acid should be close to zero here).
- Concentrations can be converted to molar concentrations and (after the sample volume has been entered) to nucleic acid quantities (method step: **process results**).

### 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 proteins in sample volumes within the microliter range with a 1 mm light path (with microliter cuvettes such as Eppendorf µCuvette G1.0 or Hellma® TrayCell).
- The following additional information on the purity of the measured proteins is displayed and can be excluded from the measuring parameters if desired:
  - absorbance wavelength spectrum of the protein
  - absorbance of the background wavelength (preset: 320 nm; the absorbance of the pure protein should be close to zero here).
- Partial turbidity correction via the **Background** parameter is preset.
- When programming the methods, the corresponding factor is imported through the simple selection of the protein from a predefined list. The factors are defined separately 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.

### Bacterial density

- Turbidity measurement to determine the bacteria density.
- Measurement at 600 nm is already preprogrammed.



The measurement of the bacterial density at 600 nm is not an absolute measurement. There are various factors that can affect the result of the measurement. Detailed information can be found on our website, [www.eppendorf.com](http://www.eppendorf.com)

## 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.

### 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 <i>Absorbance values on p. 81</i> ). Therefore, there is no need to change factors such as "50" for the calculation of dsDNA concentrations when modifying the <b>Cuvette</b> parameter.
Wavelength	Selection: 230   260   280   320   340   405   490   562   595   600 nm	Measurement wavelength: The concentration is calculated based on the absorbance measured at this wavelength. For some method groups (e.g., <b>Nucleic acids</b> and <b>Proteins direct UV</b> ), the wavelengths are preprogrammed.
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 <b>General Method Parameters/Units</b> function. Max. 7 digits.	Unit for the concentration result. In the preprogrammed methods of the <b>Routine</b> group, the selection is restricted to units that are useful for these methods.
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. For the <b>Factor</b> method group, you also can enter negative factors.
Protein	Selection: List of protein types which are stored in the <b>General Method Parameters/Proteins</b> function.	Only for the <b>Proteins direct UV</b> method group. When selecting the protein, the corresponding <b>Factor</b> parameter programmed in the <b>General Method Parameters/Proteins</b> function also will be imported from that function.

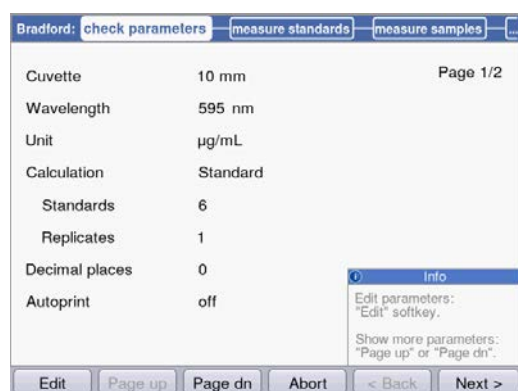
Parameter	Entry	Explanation
Standards	Value input: Number of standards. Range: 1 to 12.	Number of different standard concentrations for the evaluation with standards. For some methods the range for the number of standards is restricted to a smaller range than 1 to 12.
Replicates	Value input: Number of 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.
Show scan	Selection: on   off	Only for the <b>Nucleic acids</b> and <b>Proteins direct UV</b> method groups: Display of a restricted scan (absorbance wavelength graph at 3 nm distances) in addition to the result of the sample measurement.
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.
Background	Selection: on   off	Only for the <b>Nucleic acids</b> and <b>Proteins direct UV</b> method groups: 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. Frequently used applications: Partial correction of turbidity for measurements of nucleic acids (see background wavelength in this case: 320 nm or 340 nm).
Wavelength	Selection: 320   340 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.

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Parameter	Entry	Explanation
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 <b>print &amp; export</b> method step at the end of a measuring series.
Transmission	Selection: on   off	If the <b>Calculate Transmission</b> parameter is selected, the transmission (in %) of the sample is displayed.

## 6.4 Method procedure



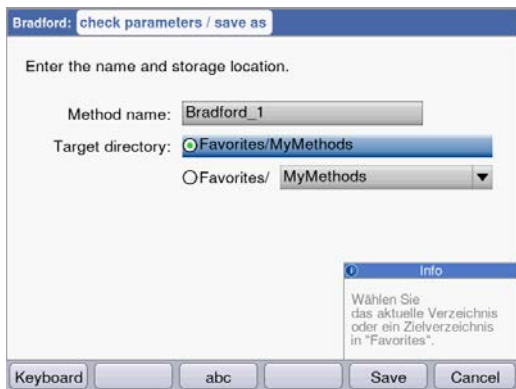
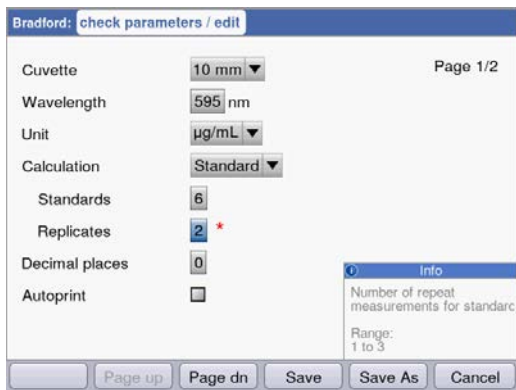
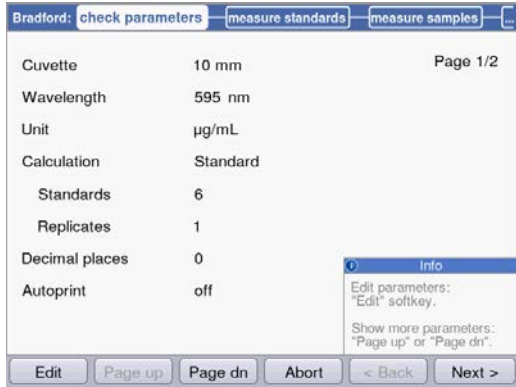
Wizard: the wizard at the top of the display will take you through the method procedure. The currently active method step is highlighted.

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
<b>check parameters</b>	Check method parameters. Carry out changes if required.
<b>measure standards</b>	Only for methods with standard evaluation: Measure and evaluate standards. Alternatively, the last saved standard evaluation can be used.
<b>measure samples</b>	Measure samples
<b>process results</b>	Only for methods of the <b>Nucleic acids</b> group: postprocess results (conversion of the concentration results).
<b>print &amp; export</b>	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]: switch between the 1 to 3 parameter pages.
- [Edit]: switch to parameter edit mode.

#### Editing mode for parameters:

Modified parameters are marked with a red star until the modification has been saved.

#### Softkeys

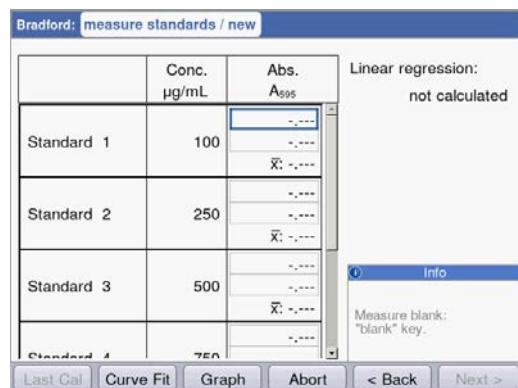
- [Save] and [Save as]: Save changes. When using [Save as] you have to rename the method. This is always the case when modifying the methods preprogrammed by Eppendorf in the **Routine** group.
- [Cancel]: exit edit mode without saving the changes.

#### 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.

You can enter the name (max. 20 characters) using the displayed keyboard ([Keyboard] softkey) or directly using the keypad (see *Entering text on p. 21*). After saving you will return to the **check parameters** display.

## 6.4.2 measure standards

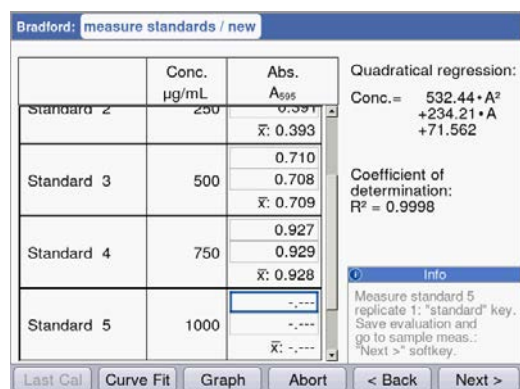


The first standard to be measured is marked on the display. After measuring the blank value (**blank** key), measure all standards (**standard** key) one by one. When measuring more than one replicate per standard, the average value for each standard is calculated and displayed automatically.

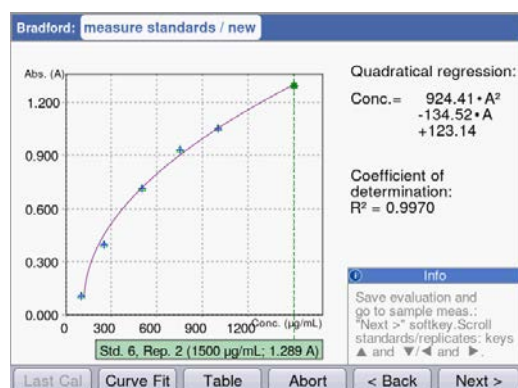
With the  $\blacktriangle$  and  $\blacktriangledown$  cursor keys, you can also select specific individual standards for measurements. Individual standards can be remeasured as well.

## Softkeys

- [Last call]: call up the last saved standard evaluation for this method in order to use it for sample measurements.
- [Curve fit]: select a method for 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. 84*).
- [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.

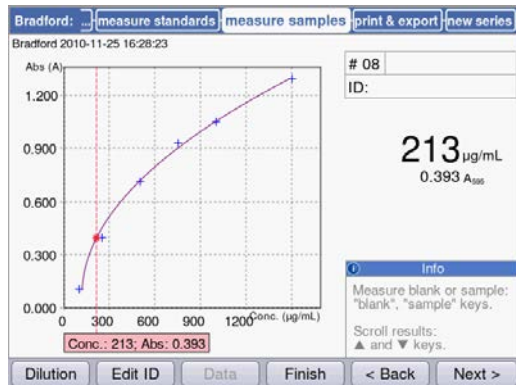
Use the  $\blacktriangle$  and  $\blacktriangledown$  cursor keys to navigate between the standards and display the results. With more than one replicate per standard, you can switch between the replicate results using  $\blacktriangle$  and  $\blacktriangledown$ . You can also select individual standards from the graphical display and measure or remeasure them.

## Softkeys

- [Table]: switch to the tabular 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. 44).

#### Softkeys

- [Dilution]: enter sample dilution.
- [Edit ID]: enter sample ID
- [Data]: display additional result data (not available for all methods).
- [Finish]: end series of measurements and return to method selection.

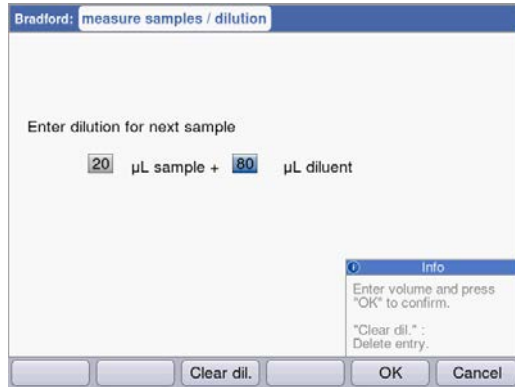


The displayed absorbance values always correspond to the directly measured values. The dilution or cuvette factor as well as background absorbances will be incorporated for the following result calculation (see *Absorbance values on p. 81*).

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### Enter dilution



The [Dilution] softkey is activated after the blank value (**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.

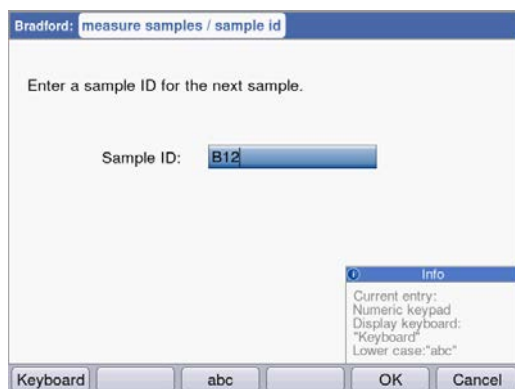
### Softkeys

- [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).

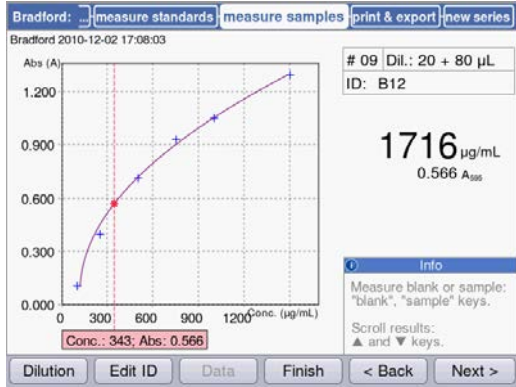
Alternatives for character input:

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

### Softkeys

- [Keyboard]: display keyboard.
- [abc]: change between upper and lower case letters when making entries using 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

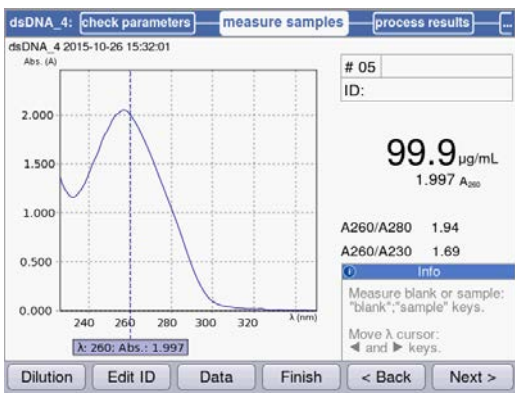


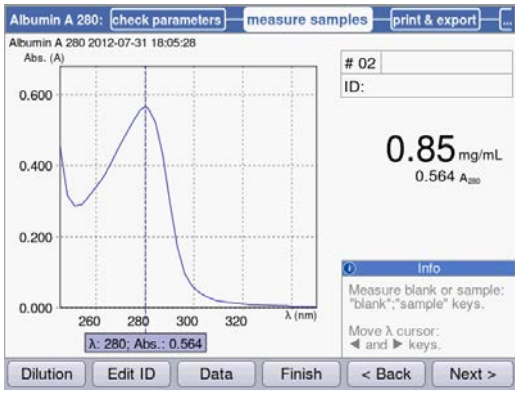


6.4.4 measure samples: result displays

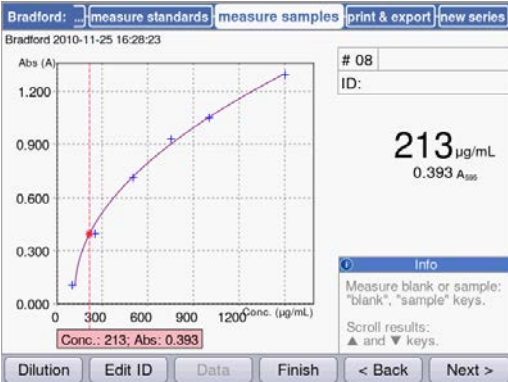
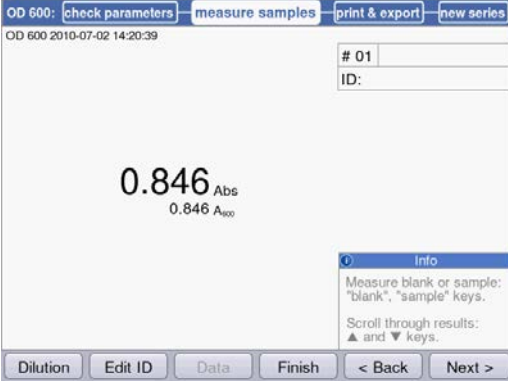
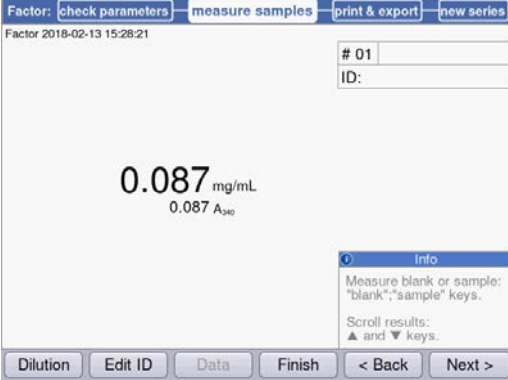
This section contains a display of typical results displays for all method groups and an overview of additional result data, which can be accessed using the [Data] softkey.

Method group	Results display	Explanation
<b>Absorbance main group</b>		
Single λ	<p>The screenshot shows the 'Single λ' results display. It features a top menu with 'check parameters', 'measure samples', 'print &amp; export', and 'new series'. Below the menu, the sample ID is '# 01'. The main result is '4.390 A<sub>340</sub>' and '0.439 A<sub>340 1 mm</sub>'. At the bottom, there are buttons for 'Dilution', 'Edit ID', 'Data', 'Finish', '&lt; Back', and 'Next &gt;'. An 'Info' box at the bottom right contains instructions: 'Measure blank or sample: "blank", "sample" keys.' and 'Scroll through results: ▲ and ▼ keys.'</p>	<p>Results display:</p> <ul style="list-style-type: none"> <li>• Absorbance at the measuring wavelength</li> <li>• Only for dilutions or with cuvettes other than 10 mm: additional display of the absorbance value before the conversion.</li> </ul>
Transmission	<p>The screenshot shows the 'Transmission' results display. It features a top menu with 'check parameters', 'measure samples', 'print &amp; export', and 'new series'. Below the menu, the sample ID is '# 01'. The main result is '29%'. At the bottom, there are buttons for 'Dilution', 'Edit ID', 'Data', 'Finish', '&lt; Back', and 'Next &gt;'. An 'Info' box at the bottom right contains instructions: 'Measure blank or sample: "blank", "sample" keys.' and 'Scroll results: ▲ and ▼ keys.'</p>	<p>Results display:</p> <ul style="list-style-type: none"> <li>• Transmission of the sample in [%]</li> <li>• Results from cuvettes with a thinner optical path length than 10 mm are marked in the results display.</li> </ul>

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Method group	Results display	Explanation
<b>Routine main group</b>		
<b>Nucleic acids</b>		<p>Results display:</p> <ul style="list-style-type: none"> <li>• Concentration result with absorbance at the measuring wavelength</li> <li>• Can be deactivated in the parameters: ratio A260/A280</li> <li>• Can be deactivated in the parameters: ratio A260/A230.</li> <li>• Can be deactivated in the parameters: scan.</li> </ul> <p>Navigate between the measuring points on the graph which are used for the result calculation with  and .</p> <p>Additional data ([Data] softkey):</p> <ul style="list-style-type: none"> <li>• Absorbance value for 280 nm.</li> <li>• Absorbance value for 230 nm.</li> <li>• Absorbance value for the background wavelength.</li> </ul>
<b>Proteins direct UV</b>		<p>Results display:</p> <ul style="list-style-type: none"> <li>• Concentration result with absorbance at the measuring wavelength</li> <li>• Can be deactivated in the parameters: scan.</li> </ul> <p>Navigate between the measuring points on the graph which are used for the result calculation with  and .</p> <p>Additional data ([Data] softkey):</p> <ul style="list-style-type: none"> <li>• Absorbance value for 260 nm.</li> <li>• Absorbance value for the background wavelength.</li> </ul>

Method group	Results display	Explanation
<b>Proteins (with reagent)</b>		<p>Results display:</p> <ul style="list-style-type: none"> <li>• Concentration result with absorbance at the measuring wavelength.</li> <li>• For evaluations with standard series: graph of the standard evaluation with plotted sample result.</li> </ul>
<b>Bacterial density</b>		<p>Results display:</p> <ul style="list-style-type: none"> <li>• Calculated result with absorbance at the measuring wavelength.</li> </ul>
<b>Basic main group</b>		
<b>Factor, standard</b>		<p>Results display:</p> <ul style="list-style-type: none"> <li>• Concentration result with absorbance at the measuring wavelength.</li> </ul>
<b>Calibration curve</b>	<p>Analog to <i>Proteins (with reagent)</i> (see above)</p>	<p>Results display:</p> <ul style="list-style-type: none"> <li>• Concentration result with absorbance at the measuring wavelength.</li> <li>• Graph of the standard evaluation with plotted sample result.</li> </ul>

## Methods

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### 6.4.5 process results

In the method procedure, two optional steps follow the sample measurement: **process results** and **print & export**.

In the **process results** step, you can convert the concentration results to molar concentrations or, after entering the volumes, into total amounts for the methods of the **Nucleic acids** group.

As in the results display, you can navigate between the sample results of the measuring series using the **▲** and **▼** cursor keys and select specific results for postprocessing.

#### More calculations

The top screenshot shows the 'process results' screen. It displays a concentration of 6.0 µg/mL and 0.120 A<sub>260</sub>. The interface includes a menu bar with options: 'measure samples', 'process results', 'print & export', and 'new series'. Below the main display, there are buttons for 'More Calc.', 'Data', 'Finish', '< Back', and 'Next >'. An 'Info' box is visible on the right side.

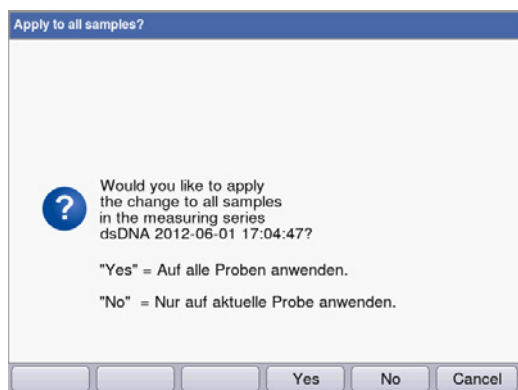
The bottom screenshot shows the 'more calc.' screen. It allows users to input 'Total volume of sample' (100 µL) and 'Molecular mass' (297 basepairs, 196 kDa). The 'Calculations' section shows results for dsDNA: 6.0 µg/mL, 0.6 µg total amount in sample, 30.7 pmol/mL, and 3.1 pmol total amount in sample. The interface also shows a 'Save' and 'Cancel' button at the bottom.

Press the [More calc.] softkey.

- After entering the molar mass (in base/base pairs or in kDa): convert the concentration result to the molar concentration.
- After entering the sample volume: calculate the total amount in the sample.
- [Save]: save changes and return to the **process results** method step.
- [Cancel]: cancel and return to the **process results** method step.



- 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, the calculation of the molar concentration is always based on the assumption that the nucleic acids are double-stranded.



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

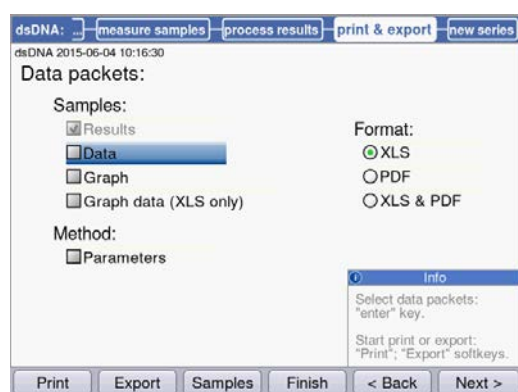
## Methods

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### 6.4.6 print & export

In the last optional method step, you can assemble data packets for all samples of a series of measurements or selected samples of a series of measurements:

- for printing on a printer
- for export to a USB stick
- for direct export to a PC using a USB cable
- for export by e-mail



#### Select data packets

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

#### Select the format

- XLS: export as an Excel spreadsheet.
- PDF: export as a PDF or print.

#### Softkeys

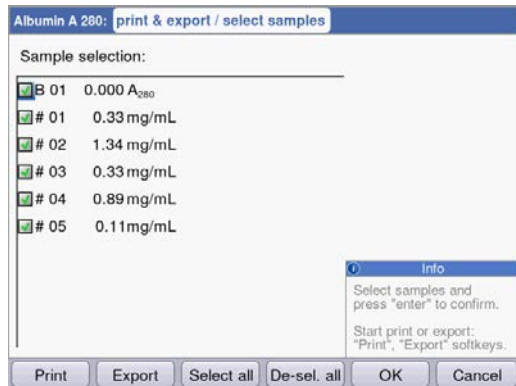
- [Print]: start printing.
- [Export]: start export.
- [Sample]: select individual sample results.

#### Select data packets

Results	Primary result data; cannot be selected because it is always transferred.
Data	Additional result data that can be displayed during the measurement by pressing the [Data] softkey.
Graph	Absorbance wavelength spectrum.
Parameters	Method parameters
Standards/results	Result 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

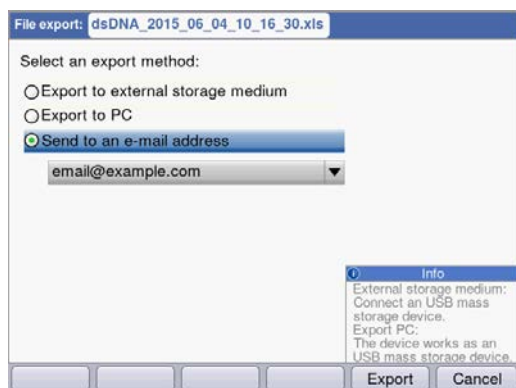
- Press the [Samples] softkey to call up the sample selection.
- Use the cursor keys for navigating and confirm with **enter**.

#### Softkeys

- [Select all]: select all samples
- [De-Sel. all]: cancel selection.

### Start the export

The data will be transferred as an Excel (.xls) file or as a PDF. Excel files can be read with Excel 97 or later versions. 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 e-mail: send data to an e-mail address.

### Export to USB stick

1. Connect a FAT 32-formatted USB stick to USB port **4** (see *Product overview on p. 13*).
2. Press [Export] to start exporting to an external storage medium.

### Export to a PC

Requirement for the PC operating system: Windows XP, SP2 or higher.

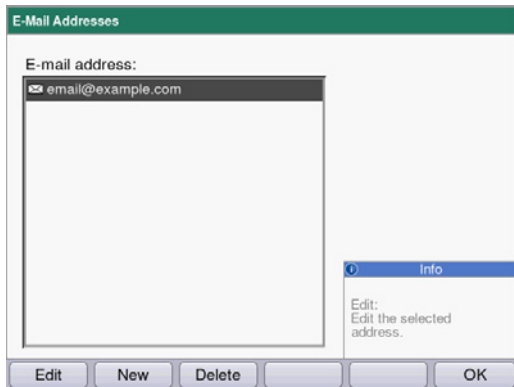
1. Insert the USB cable into port **8** of the device (see *Product overview on p. 13*) and connect it to the PC.
2. Prior to beginning a new export make sure that any data that has been exported previously has been saved to the PC hard drive. Otherwise, the new export will overwrite the data.
3. Press [Export] to start exporting to the PC.
4. The exported data packet will be displayed on your PC as a removable drive named "eppendorf". Open the file on this drive and save it to the hard drive.

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### Export to an e-mail address

1. Select an e-mail address from the list or select "Edit" to set up a new e-mail address.
2. Press [Export] to start sending the export to an e-mail address.



### Edit e-mail addresses

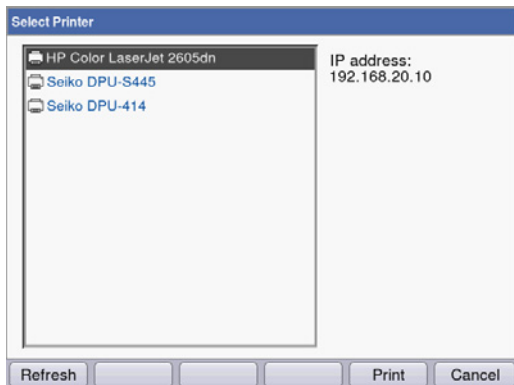
- Select "Edit" from the drop-down list and confirm with **enter**.  
A window in which the e-mail addresses can be edited opens.
- [Edit]: edit an e-mail address.
- [New]: create a new e-mail address.
- [Delete]: delete an e-mail address.

### Start printing

The data can be printed on a printer in the network or on a connected USB printer.



If the device is connected to a network, all compatible printers in the network are detected and displayed automatically. If no connection to the network exists, only a connected USB printer can be selected.

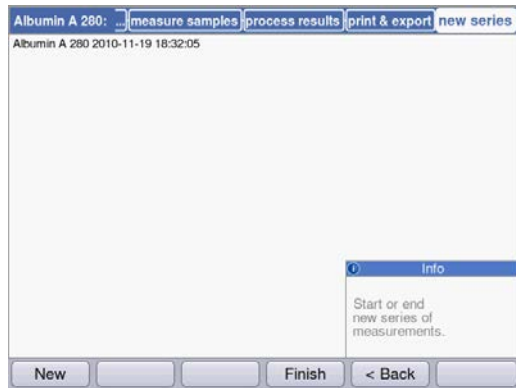


1. Select a printer.
2. Press [Print] to print the data.

## 6.4.7 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 the **new series** method step
- [New] softkey: call up the **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.

**Methods**

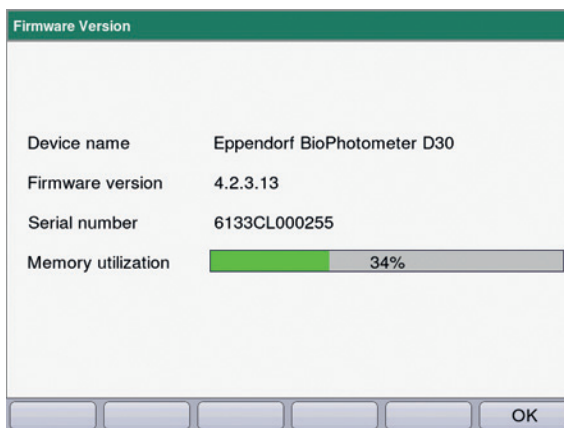
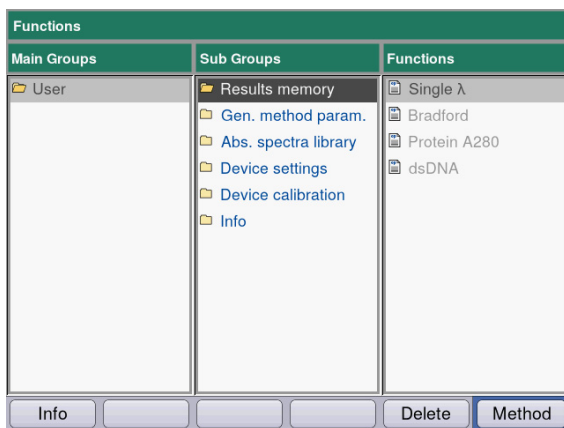
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## 7 Functions

### 7.1 Functions of the *User* main group

The **function** key or [Function] softkey opens a menu with functions such as device settings and where you can access 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 in the method selection, you use the cursor keys to navigate. First, you select the desired subgroup and then the desired function in the right column. Press **enter** to open the function.



[Info] softkey:

- Firmware version
- BioPhotometer D30 serial number
- Current memory usage

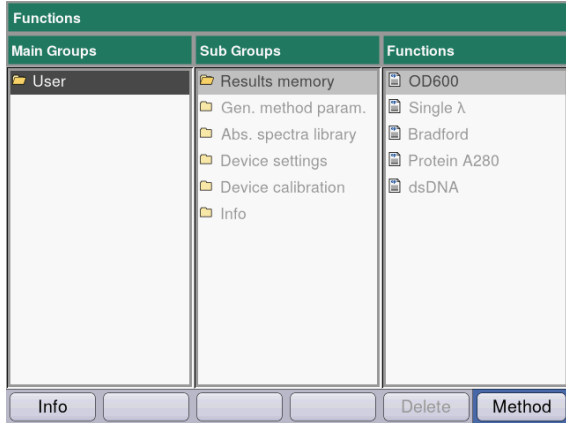
## Functions

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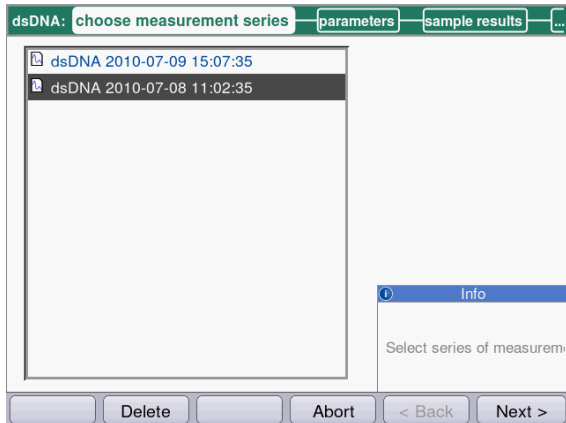
Tab. 7-1: Overview of the functions

Subgroup	Explanation
<b>Results memory</b>	<p>Displays saved results. The results can be accessed structured according to methods and series of measurements and can be printed, exported and deleted directly from the memory. It is possible to delete individual series of measurements, all series of measurements of a method or the entire results memory.</p> <ul style="list-style-type: none"> <li>▶ To delete the method and all associated series of measurements, press the <b>Delete</b> softkey.</li> <li>▶ Confirm with <b>enter</b>.</li> </ul>
<b>General method parameters</b>	<p>Parameters which are used for different methods in common are stored centrally in the <b>Functions</b> area. Factory-set parameters cannot be deleted. Parameters created by the user can be changed freely. In the <b>check parameters</b> method step, the comprehensive parameters can be easily selected using drop-down menus.</p> <ul style="list-style-type: none"> <li>• <b>Proteins:</b> parameters for methods of the <b>Proteins direct UV</b> group</li> <li>• <b>Units:</b> units for concentration results that can be used for many methods.</li> </ul>
<b>Absorbance spectra library</b>	<p>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.</p>
<b>Device settings</b>	<p>Editable device settings, e.g., language.</p>
<b>Device calibration</b>	<ul style="list-style-type: none"> <li>• Option for inspecting the photometer. An Eppendorf filter set is required for this.</li> </ul>
<b>Info</b>	<p>Open-source licenses.</p>

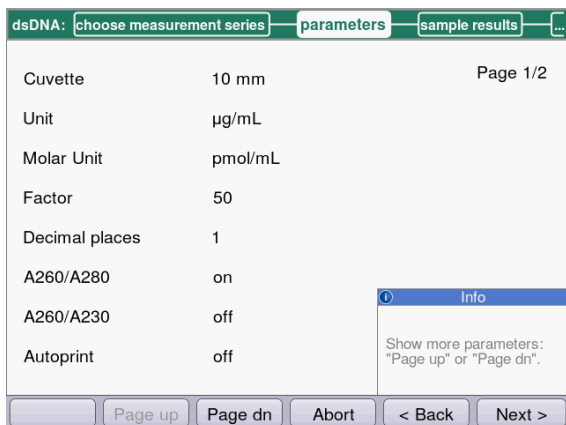
### 7.1.1 Results memory



- ▶ In the right column, select the method for which you would like to call up saved results.
- ▶ To delete the method and all associated series of measurements, press the **Delete** softkey.
- ▶ Confirm with **enter**.



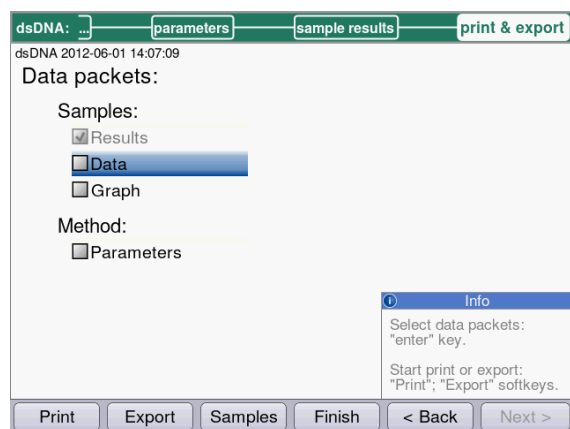
- ▶ Select the desired series of measurement with the cursor keys.
- ▶ To delete the method and all associated series of measurements, press the **Delete** softkey.
- ▶ Confirm with **enter**.



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.

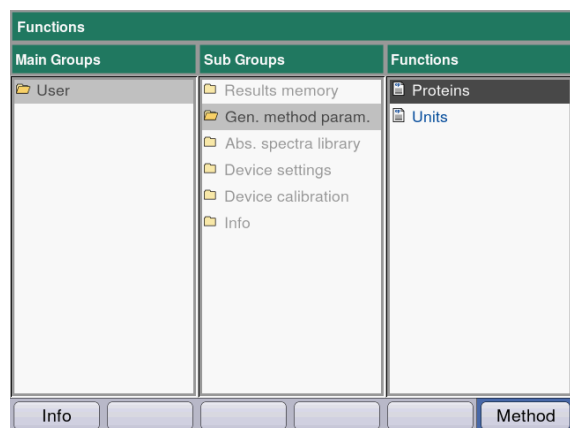
## Functions

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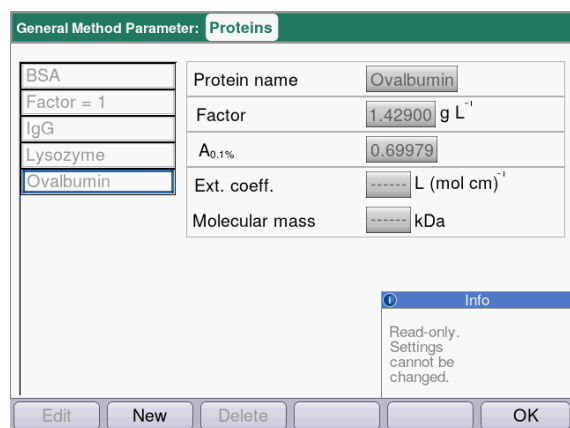


- ▶ If you would like to print or export results, select the data packets. The procedure for printing and exporting and the meaning of the function keys correspond to the **print & export** method step.

### 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, the parameter groups for different proteins are subsumed and stored respectively under a name. This name can be used to import the desired parameter group into the method program when editing a protein method (**Proteins direct UV** method group).

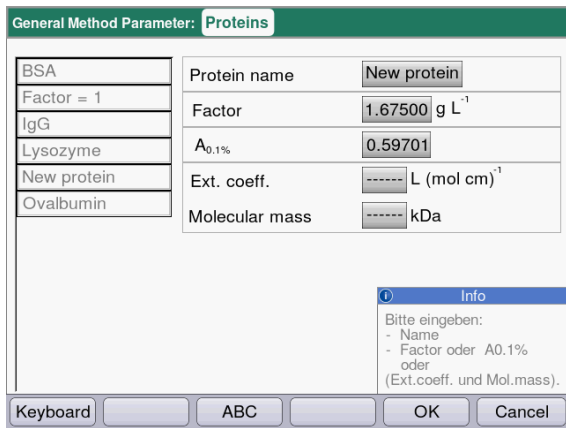
The factory-set proteins are read-only and cannot be edited or deleted.

Display:

- left: name of the protein. Select via ▲ and ▼.
- right: associated parameters

#### Softkeys

- [Edit]: edit selected parameter group.
- [New]: create new parameter group.
- [Delete]: delete selected parameter group.
- [OK]: return to function selection.

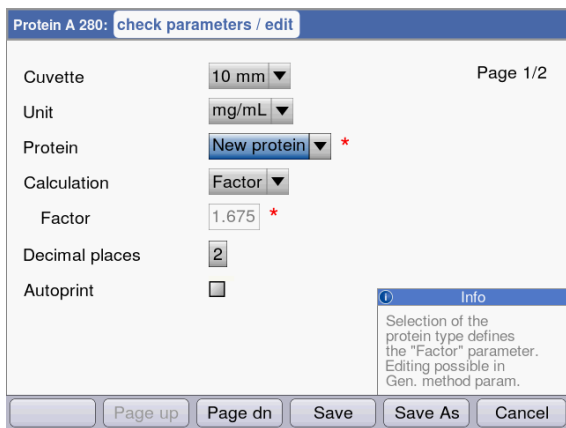


- ▶ To edit a parameter group, use ▲ and ▼ to select the parameter which you would like to edit.
- ▶ Confirm with **enter**.

**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 **Proteins direct UV** method group, you can access the entries in **General Method Parameter**:



Select the name of the protein in order 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
<b>Proteins</b>	When selecting a protein while programming a method of the <b>Proteins direct UV</b> group, these parameters are loaded into the method parameters. The factory-programmed parameters are read-only and cannot be edited or deleted.
<ul style="list-style-type: none"> <li>• Protein name</li> <li>• Factor</li> <li>• A<sub>0.1%</sub></li> <li>• Ext.coeff.</li> <li>• Molecular mass</li> </ul>	To define a factor for calculating the concentration from the absorbance, you can enter the following data in addition to the name and wavelength: Factor <b>or</b> A <sub>0.1%</sub> <b>or</b> absorbance coefficient and molar mass.

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Parameter	Explanation
<b>Units</b>	You can select a unit from all available units when programming method parameters. Units used in preprogrammed methods are gray and cannot be deleted.
• 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

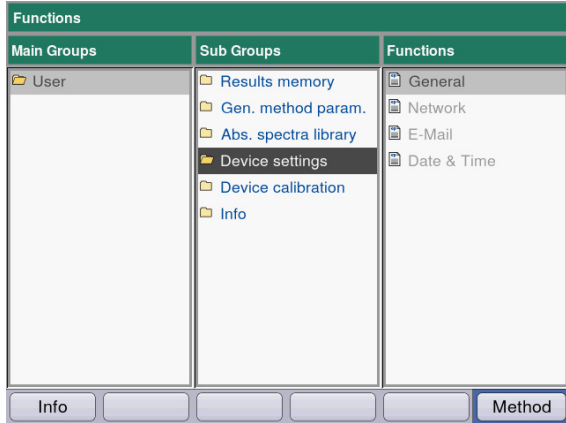
The screenshot shows the 'Functions' menu with 'Abs. spectra library' selected. Below it, the 'Absorbance Spectra Library: dsDNA' window is open, displaying a graph of Absorbance (A) versus wavelength ( $\lambda$  in nm). The graph shows a peak at approximately 260 nm with an absorbance of about 0.9. The x-axis ranges from 240 to 340 nm, and the y-axis ranges from 0.000 to 0.800. Below the graph are buttons for 'Export', 'Print', and 'OK'.

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

#### Softkeys

- [Export] and [Print]: export to a USB stick, to a PC (using a USB cable) or print (see *print & export on p. 44*).
- [OK]: return to function selection.

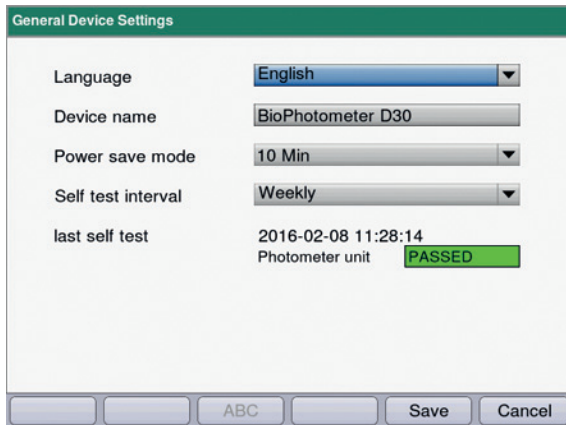
### 7.1.4 Device settings



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, Japanese\*).
- Device name
- Set the time interval for activating power saving mode.
- Set the interval for the automatic self test that is performed after the device is switched on.
- Information on the last self test is displayed.

\*) If the language is switched, e.g., to Japanese, the font changes. This may cause parts of the text not to be displayed correctly.

- ▶ Switch the device off and back on. The languages will be displayed correctly after the restart.

#### Softkeys

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

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### Network Settings

Ask your network administrator which settings are required.

- Selection whether IP settings are to be made automatically via DHCP. The IP settings can also be entered manually.
  - IP address
  - Subnet mask
  - Standard gateway
- Selection whether DNS settings are to be made automatically via DHCP (only available if IP settings are obtained automatically via DHCP). The following DNS settings can be entered manually:
  - primary DNS server
  - secondary DNS server

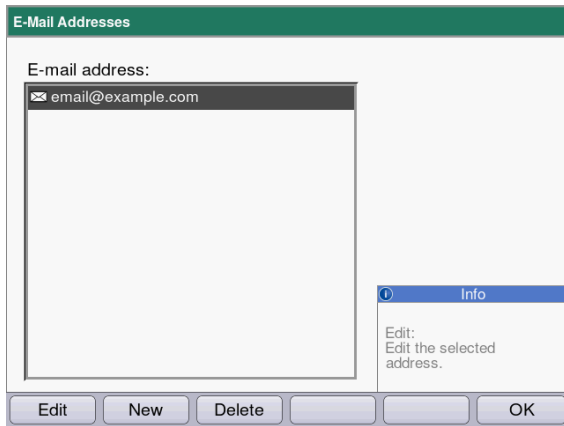
### Softkeys

- [MAC Info]: information on the 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 e-mail address: enter the device name.
- Use SMTP authentication: if authentication is required, a user name and password need to be defined.
- Recipient e-mail address: list of e-mail addresses.

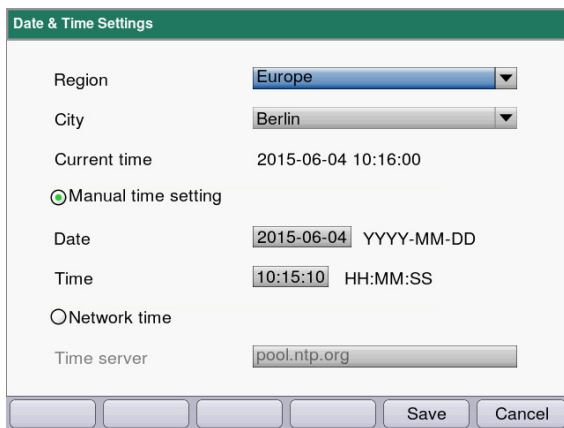


**Edit e-mail addresses**

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

**Softkeys**

- [Edit]: edit an e-mail address.
- [New]: create a new e-mail address.
- [Delete]: delete an e-mail address.



**Date and Time Settings**

- Select a region.
- Select a city.
- Display of the current time
- Manual time setting: enter date and time.
- Network time  
Time server: enter the desired time server.

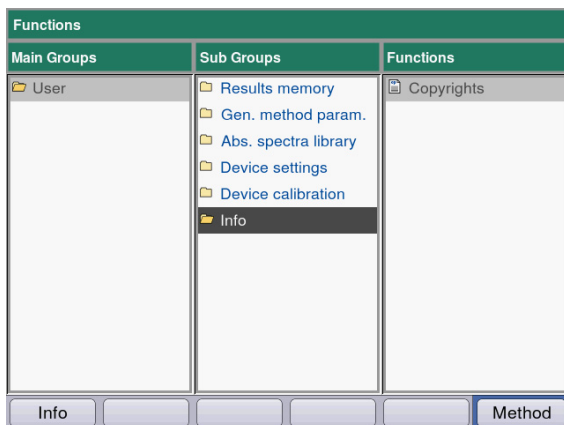
**Softkeys**

- [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. 61*).

**7.1.6 Info**



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

**Functions**

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## 8 Maintenance

### 8.1 Cleaning

---



**DANGER! Electric shock due to the ingress of liquid.**

- ▶ Switch off the device and disconnect it from the mains/power line before starting cleaning or disinfection.
  - ▶ 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 once it is completely dry, both inside and outside.
- 



**NOTICE! Corrosion due to aggressive cleaning agents and disinfectants.**

- ▶ Do not use any corrosive cleaning agents, aggressive solvents or abrasive polishes.
  - ▶ Do not incubate the accessories in aggressive cleaning agents or disinfectants for longer periods.
- 

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.

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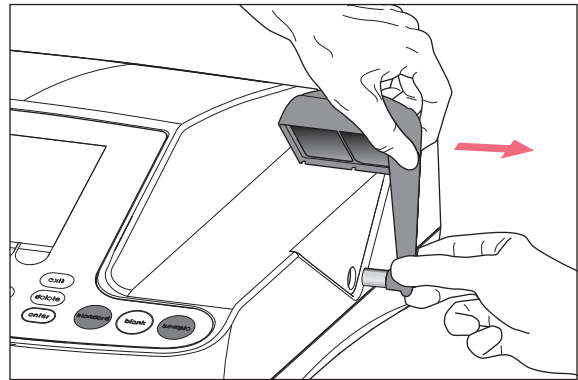
**8.1.1 Cleaning the cuvette shaft cover**

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 due to the ingress of liquid.**

- ▶ Switch off the device and disconnect it from the mains/power line before starting cleaning or disinfection.
  - ▶ 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 once it is completely dry, both inside and outside.
- 

1. Clean the device with a mild cleaning agent before the disinfection (see *Cleaning on p. 59*).
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 disassembly and assembly (see *Cleaning the cuvette shaft cover on p. 60*).
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. 77*).
- Perform the check at approx. 20 °C. Avoid temperature variations (e.g., due to open windows).
- Remove the filter from the filter box only briefly and protect it against contamination or damage to the filter surfaces.
- Protect the filters against dust, heat, liquids and aggressive vapors.
- When checking the photometer unit: the label of the filter used shows to the front.
- The cuvette shaft is free from contamination.

### **8.3.1 Checking the photometer unit**

Eppendorf offers a filter kit for checking the photometric accuracy and the wavelength systematic error. The kit contains one blank filter A0 and 3 filters A1, A2 and A3 for checking the photometric accuracy as well as 2 filters for checking the wavelength systematic error (260 nm, 280 nm). The filter absorbances are measured against the blank filter A0. In addition to the information on the accuracy, you also receive information on the precision: average value and variation coefficient (CV value) are calculated from the sets of 15 measurements per wavelength.

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

**Maintenance**

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If you would like to document the values, you can print or export them after the measurement. A maximum of 12 checks will be stored. When the storage medium is full, the values of the oldest check will be overwritten.

**BioPhotometer D30 reference filter set**



Function : Device calibration/Photometer unit

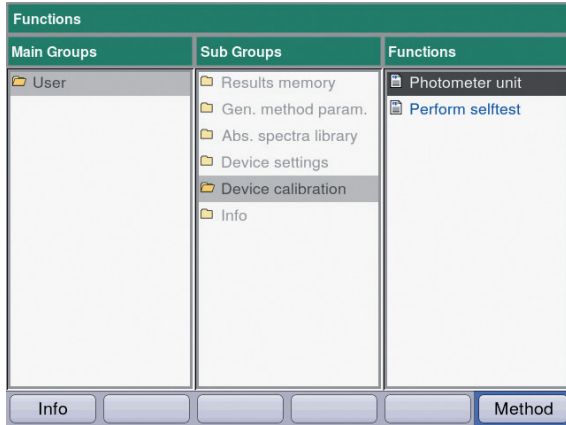
Order No./Best. Nr.: 6133 928.004

Set No./Satz Nr.: 840

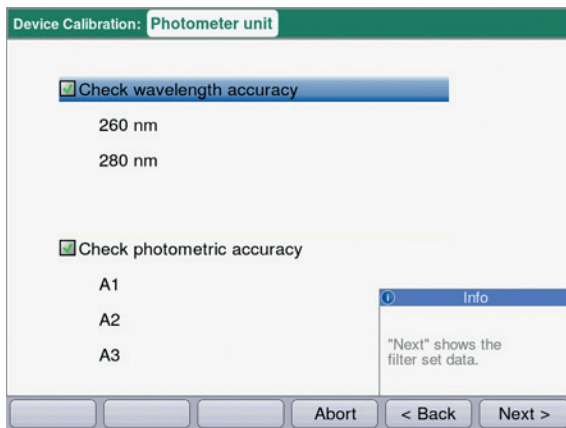
Limits measured against Blank A 0 at approx. 20°C Grenzwerte gemessen gegen Blank A 0 bei ca. 20°C						
SN: 6131	914.840	916.840	917.840	921.840	922.840	923.840
Filter Type	Blank A 0	Sample 260 nm	Sample 280 nm	Sample A 1	Sample A 2	Sample A 3
Limiting values (A)/Grenzwerte (E)						
260 nm	0.000	1.165-1.352	--	0.157-0.181	0.904-0.960	1.760-1.869
280 nm	0.000	--	0.990,1.224	0.152-0.176	0.901-0.956	1.719-1.826
320 nm	0.000	--	--	0.147-0.171	0.905-0.961	1.665-1.768
405 nm	0.000	--	--	0.144-0.168	0.929-0.986	1.586-1.684
562 nm	0.000	--	--	0.143-0.167	0.929-0.987	1.427-1.515
595 nm	0.000	--	--	0.142-0.166	0.925-0.982	1.397-1.484
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 - 600 nm	≤ 3.0 %		≤ 3.0 %		≤ 2.0 %	≤ 3.0 %
Filter auf NIST® rückführbar / Filter traceable to NIST®						
<p><u>Wavelength and photometric characterization of filters:</u> All characterizations are performed on a Cary 100 Bio reference UV/Vis spectrophotometer, serial number EL 99023107. The instrument is requalified regularly by the manufacturer, and is confirmed and documented to perform within manufacturer's specifications.</p> <p><u>Wellenlängen- und photometrische Bestimmung der Filter:</u> Alle Messungen werden auf einem Cary 100 Bio Referenz UV/Vis Spektrophotometer, Seriennummer EL 99023107 durchgeführt. Dieses Instrument wird regelmäßig vom Hersteller requalifiziert und die spezifikationsgemäße Funktion dokumentiert.</p>						
				6133 928.028-03		
				21.12.2017 ----- Signature Unterschrift		

Fig. 8-1: Inside lid of the filter box (example)

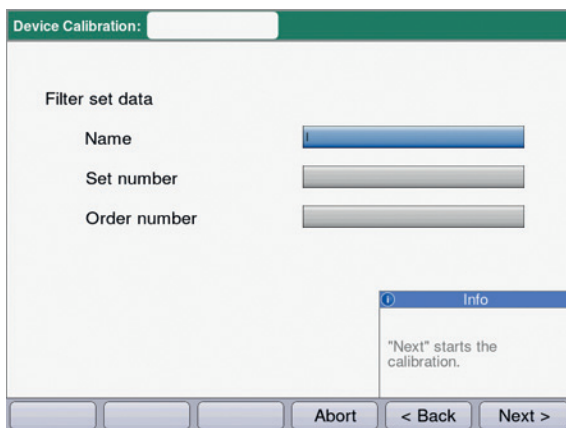
### 8.3.1.1 Checking the photometric accuracy



1. In the **Device calibration** group, select the **Photometer unit** function and confirm with **enter**.



2. Select whether you want to check the wavelength systematic error (wavelength accuracy), the photometric accuracy or both. Confirm with **enter**.
3. Press [Next >] to go to the next step.



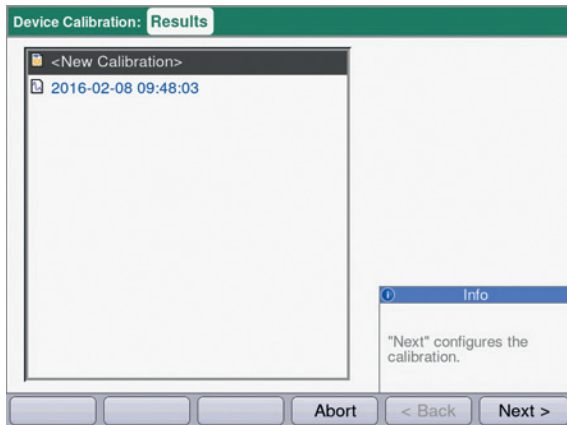
4. Complete the entry fields. All entries are optional.
5. Press [Next >] to go to the next step.

## Maintenance

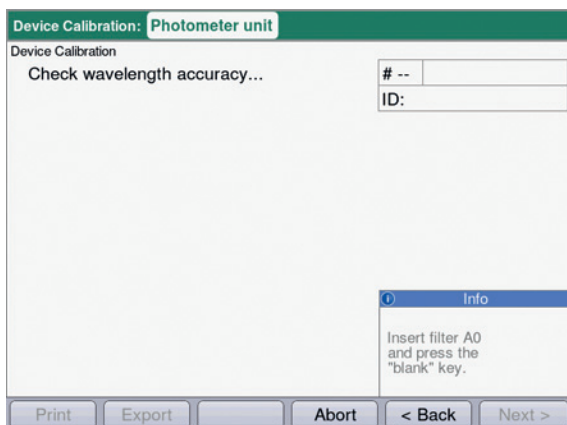
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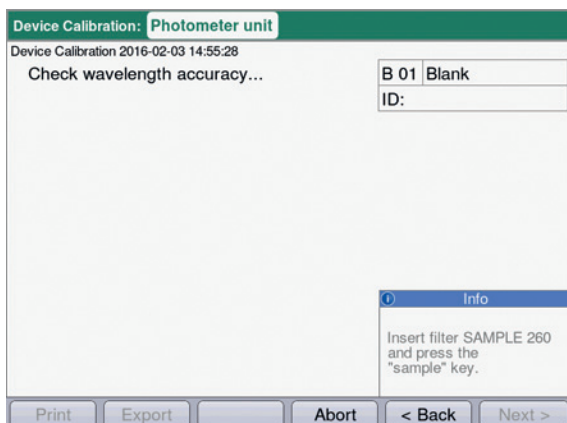
- If calibration is performed for the first time, step 6 is omitted.
- If a calibration has already been performed, the results of the last calibration will be displayed.



6. Select <New Calibration> and press [Next >] to start the calibration.



7. Follow the instructions in the *Info* box and measure blank filter A0 first.



8. After measuring the blank value A0, start with the first test filter.  
The Info box displays the expected test filter (here: SAMPLE 260).

Device Calibration: **Photometer unit**  
Device Calibration 2016-02-08 11:24:19  
Check photometric accuracy... # 05  
ID: SAMPLE A3

Wavelength	Mean	CV
260 nm	1.904 A	0.2 %
280 nm	1.835 A	0.2 %
320 nm	1.741 A	0.2 %
405 nm	1.655 A	0.2 %
562 nm	1.484 A	0.1 %
595 nm	1.456 A	0.4 %

Info  
Select results:  
▲ and ▼ keys.

Print Export Finish < Back Next >

9. Results display after measuring all 3 test filters for testing the photometric accuracy. Use the ▲ and ▼ keys to view the results for the different test filters again.

**Softkeys**

- [Finish]: finish the check.
  - [Export]: export results to PDF.
  - [Print]: print results.
10. Compare the average values and variation coefficients with the supplied table. If the measured values do not agree with the permitted range of values, contact Eppendorf Service.

**8.3.2 Device self test**

You can set the frequency of the automatic self test (duration approx. 1 minute) with the **Device settings** function (see *Device settings on p. 55*). The factory setting for the **self-test interval** is "Weekly".

The self test checks the following:

- Verification of the detector
    - Determination of the random error of the available wavelengths
  - Verification of the light source
    - Verification of the maximum available energy of the light source and quality of the light transmission through the device
    - Determination of the random error of a signal at 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
- In the **Device calibration** group, select the **Perform selftest** function and confirm with **enter**. After completion of the self test, the display shows **PASSED**. If **FAILED** is displayed, the self test has failed. If this error cannot be corrected (see *Error messages on p. 69*), contact the authorized service.

**Maintenance**

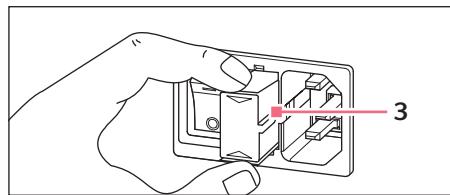
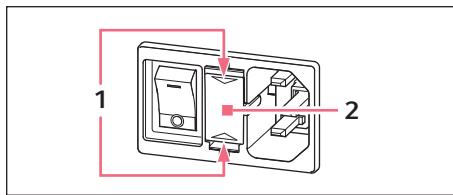
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## 8.4 Replacing fuses

**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. Observe the information on the decontamination certificate. You can find it as a PDF document on our webpage ([www.eppendorf.com/decontamination](http://www.eppendorf.com/decontamination)).
2. Decontaminate all the parts you would like to dispatch.
3. Include the fully completed decontamination certificate in the package.

**9 Troubleshooting**  
**9.1 General errors**

Error	Possible cause	Remedy
<p>Measuring results are imprecise.</p>	<ul style="list-style-type: none"> <li>• Reagent is past its shelf life.</li> <li>• Reagent has not been prepared properly.</li> <li>• The pipetting is not correct.</li> <li>• Incubation procedure before measurement is incorrect.</li> <li>• The cuvette is contaminated.</li> <li>• The cuvette is not filled completely with measuring solution, and it contains bubbles.</li> <li>• Turbidity of the measuring solution.</li> <li>• Photometer is drifting.</li> <li>• Cuvette shaft is dirty.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Ensure that the reagent is still within its shelf life and properly prepared.</li> <li>▶ If required, use clean demineralized water of an adequate quality for preparation.</li> <li>▶ Ensure that the pipette is calibrated and pipetting is being performed correctly.</li> <li>▶ If the method procedure requires incubation before the measurement, ensure that the temperature and time for incubation are correctly observed.</li> <li>▶ 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.</li> <li>▶ If the cuvette window has become soiled from fingerprints, wipe it clean using a lint-free lab cloth soaked in ethanol or isopropanol.</li> <li>▶ Ensure that the required minimum volume of the cuvette for a measurement is reached and that no bubbles are in the measuring solution.</li> <li>▶ Centrifuge the turbid measuring solutions that contain particles and use the clear supernatant.</li> <li>▶ Contact Eppendorf Service.</li> <li>▶ Observe the ambient conditions.</li> <li>▶ Prevent temperature changes.</li> <li>▶ Clean the cuvette shaft .</li> </ul>

**Troubleshooting**

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Error	Possible cause	Remedy
<p>Measuring results are not correct.</p>	<ul style="list-style-type: none"> <li>• The method has not been programmed correctly.</li> <li>• The standard solution has not been prepared correctly.</li> <li>• The absorbance of the reagent is drifting.</li>   <li>• The cuvette is not positioned correctly.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Ensure that the method parameters are entered correctly.</li> <li>▶ Ensure that the correct standard is used and that the measuring solution for the standard is prepared correctly.</li> <li>▶ 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.</li> <li>▶ Position the cuvette in the cuvette shaft so that the optical window points towards the direction of the light path.</li> <li>▶ Photometry light path: from back to front</li> </ul>

## 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.

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Self test failed.	<ul style="list-style-type: none"> <li>• Cuvette shaft cover was open during self test.</li> <li>• The cuvette shaft was not empty during the self test.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Repeat the self test with empty cuvette shaft and the cuvette shaft cover closed.</li> </ul>
	<ul style="list-style-type: none"> <li>• Device is faulty.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Contact Eppendorf Service.</li> </ul>
File export failed.	During data export: <ul style="list-style-type: none"> <li>• USB stick improperly formatted or faulty.</li> <li>• USB stick removed from the device too early (during the export).</li> </ul>	<ul style="list-style-type: none"> <li>▶ Reformat or replace the USB stick.</li> <li>▶ Reconnect the USB stick and repeat the export.</li> </ul>
Failed to initialize printer.	<ul style="list-style-type: none"> <li>• Printer not connected or switched off.</li> <li>• Printer not configured correctly.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Connect the printer and switch it on.</li> <li>▶ Reconfigure the printer.</li> </ul> For a correct configuration of the printer settings refer to the installation description (see <i>Connecting the printer to the USB port on p. 16</i> ).
Blank measurement: An intensity on a pixel that influences the main, auxiliary or scan wavelength is too low.	<ul style="list-style-type: none"> <li>• The absorbance of the blank solution used for the blank measurement is too high.</li> <li>• Incorrect or turbid blank solution.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Check the blank solution and remeasure the blank if required.</li> </ul>
The entered name is not valid.	<ul style="list-style-type: none"> <li>• Error when entering the name. Different causes are possible. For the precise cause please see the information in the help box.</li> </ul>	<ul style="list-style-type: none"> <li>▶ See information in the help box.</li> </ul>
A method (or folder, protein, unit) with this name already exists.	<ul style="list-style-type: none"> <li>• The name under which the method was saved has already been used for a different method in the same folder.</li> <li>• The message also appears after edition names already given to a folder or to a protein or a concentration unit (under <b>General Method Parameter</b>).</li> </ul>	<ul style="list-style-type: none"> <li>▶ Assign a different name.</li> </ul>

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<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
The following parameter values are not defined in <b>General Method Parameter</b> :	<ul style="list-style-type: none"> <li>When opening a method with parameters which access <b>General Method Parameter</b>, the system determined that at least one parameter (protein, unit) does not exist there anymore, so probably has been deleted.</li> </ul>	<ul style="list-style-type: none"> <li>Select a different parameter from the existing list. If necessary, program a new list entry in <b>General Method Parameter</b> in order to be able to use it when programming a method.</li> </ul>
The value of the parameter marked with * is not defined in the Gen. Param. Please correct the parameter.	<p>This error message appears when editing method parameters.</p> <ul style="list-style-type: none"> <li>Parameter in <b>General Method Parameter</b> is not defined.</li> </ul>	<ul style="list-style-type: none"> <li>Select a different parameter from the existing list. If necessary, program a new list entry in <b>General Method Parameter</b> in order to be able to use it when programming a method.</li> </ul>
The entered standard concentrations are not monotonically increasing resp. monotonically falling. Correct the standard concentrations.	<ul style="list-style-type: none"> <li>See the error text.</li> </ul>	<ul style="list-style-type: none"> <li>Enter the standard concentrations so that the first standard receives the lowest concentration and the other standard concentrations form an increasing sequence.</li> </ul>
At least two of the entered standard concentrations are identical. Correct the standard concentrations.	<ul style="list-style-type: none"> <li>See the error text.</li> </ul>	<ul style="list-style-type: none"> <li>Enter the standard concentrations so that the first standard receives the lowest concentration and the other standard concentrations form an increasing sequence.</li> </ul>
The measured values are not strictly monotonous!	<ul style="list-style-type: none"> <li>Error when measuring a standard series: The measured absorbance values of the standard series are not continuously increasing or decreasing.</li> </ul>	<ul style="list-style-type: none"> <li>Repeat the standard measurements or delete the single, incorrectly measured standard result.</li> </ul>
The ID cannot be set.	<ul style="list-style-type: none"> <li>Error when entering the sample ID. Different causes are possible. For the precise cause please see the information in the help box.</li> </ul>	<ul style="list-style-type: none"> <li>See information in the help box.</li> </ul>
The dilution cannot be set.	<ul style="list-style-type: none"> <li>Error when entering the dilution. Different causes are possible. For the precise cause please see the information in the help box.</li> </ul>	<ul style="list-style-type: none"> <li>See information in the help box.</li> </ul>

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
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.	<ul style="list-style-type: none"><li>• The number of measurements in one measuring series is limited to 99.</li></ul>	<ul style="list-style-type: none"><li>▶ Start a new series of measurement after maximally 99 measurements.</li></ul>

## Troubleshooting

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### 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.	<ul style="list-style-type: none"> <li>No usable result was returned during the evaluation of a standard curve using the "spline interpolation", "quadratic regression" or "cubic regression" <b>Curve Fit</b> procedures.</li> </ul>	<ul style="list-style-type: none"> <li>Select a different <b>Curve Fit</b> procedure.</li> </ul>
Some absorbance values for secondary wavelengths are too high or are not displayed.	<ul style="list-style-type: none"> <li>For at least one secondary wavelength, the absorbance exceeded the measuring range.</li> <li>Secondary wavelengths are not needed for calculating the concentration result. They are used for different purposes. For example, <b>dsDNA</b> method: absorbance at 280 nm for the calculation of <b>ratios 260/280</b>.</li> <li>Turbidity of the measuring solution</li> <li>Measurements at the limits of the photometric measuring range.</li> </ul>	<ul style="list-style-type: none"> <li>If the absorbance values of the secondary wavelengths are relevant: Dilute the sample or remove the turbidity via centrifugation and repeat the measurement.</li> </ul>
The result is outside the range of the standard concentrations.	<ul style="list-style-type: none"> <li>For methods with evaluation via standard curves (nonlinear evaluation method): The sample result is up to 5 % outside of the standard concentration range.</li> </ul>	<ul style="list-style-type: none"> <li>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).</li> </ul>
The coefficient of determination is <0.8.	<ul style="list-style-type: none"> <li>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.</li> <li>Turbidity of the measuring solution.</li> <li>Measurements at the limits of the photometric measuring range.</li> </ul>	<ul style="list-style-type: none"> <li>Accept the result of the standard evaluation or remeasure the standards.</li> <li>Make sure the measuring solutions are clear.</li> </ul>

Problem	Cause	Solution
The coefficient of determination for the regression evaluation of the standard series is < 0.8.	<ul style="list-style-type: none"> <li>• 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.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Use the sample results with the reservation mentioned or repeat the measurement of the standard series and samples.</li> </ul>
Scan: Some of the measured absorbances are too high and are not displayed.	<ul style="list-style-type: none"> <li>• For at least one scan wavelength, the absorbance exceeded the measuring range.</li> <li>• Turbidity of the measuring solution.</li> <li>• Measurements at the limits of the photometric measuring range.</li> </ul>	<ul style="list-style-type: none"> <li>▶ If the non-displayed areas of the scan are relevant: Dilute the sample or remove the dilution via centrifugation and repeat the measurement.</li> </ul>
Absorbance at the measuring wavelength is too high.	<ul style="list-style-type: none"> <li>• Turbidity of the measuring solution.</li> <li>• Optical surfaces of the cuvette are soiled.</li> <li>• Cuvette has been inserted into the cuvette shaft facing the wrong direction.</li> <li>• Too high absorbance of measuring solution.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Measure again considering the possible causes.</li> </ul>
The calculated result is negative.	<ul style="list-style-type: none"> <li>• Measuring solution not prepared correctly.</li> <li>• The incorrect factor has been entered (wrong algebraic sign).</li> </ul>	<ul style="list-style-type: none"> <li>▶ Measure again considering the possible causes.</li> </ul>
The result has more than 6 pre-decimal places.	<ul style="list-style-type: none"> <li>• Very high sample concentration.</li> <li>• Concentration unit does not match the expected range of the sample concentrations.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Dilute sample and measure again.</li> <li>▶ Change the concentration unit (Parameter <b>Unit</b>) and measure again.</li> </ul>
The result is more than 5 % outside of the standard concentration range.	<ul style="list-style-type: none"> <li>• For methods with evaluation via standard curves (nonlinear evaluation method): The sample result is more than 5 % outside of the standard concentration range.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Remeasure the sample under conditions under which the result is within the range of the standard concentrations (dilute sample, modify standard concentrations and remeasure).</li> </ul>
<ul style="list-style-type: none"> <li>• Calculation not possible because of division by zero. Absorbance result is zero.</li> <li>• Calculation error. Division by zero.</li> </ul>	<ul style="list-style-type: none"> <li>• 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.</li> </ul>	<ul style="list-style-type: none"> <li>▶ Check the reagents and samples used and repeat the measurement.</li> </ul>

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## 10 Transport, storage and disposal

### 10.1 Transport

► Use the original packing 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 packing	-25 °C – 55 °C	25 % – 75 %	70 kPa – 106 kPa
Without transport packing	-5 °C – 45 °C	25 % – 75 %	70 kPa – 106 kPa

### 10.3 Disposal

If the product needs to be disposed of, the relevant legal regulations must 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 marking:



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 $\pm$ 10 %, 50 Hz to 60 Hz
Overvoltage category	II
Degree of pollution	2
Power consumption	Maximum power consumption according to name plate: 25 W Approx. 15 W during operation Approx. 5 W with the display dimmed
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 $\times$ 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	5.4 kg
Dimensions	Width: 295 mm Depth: 400 mm Height: 150 mm
Space required	Width: 500 mm (with thermal printer: 750 mm) Depth: 500 mm

**Technical data**

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**11.4 Photometric properties**

Measuring principle	Single beam absorption photometer with reference beam
Light source	Xenon flash lamp
Monochromator	Holographic aberration-corrected concave grating
Beam receiver	CMOS photodiodes
Wavelengths	230 nm, 260 nm, 280 nm, 320 nm, 340 nm, 405 nm, 490 nm, 562 nm, 595 nm, 600 nm
Wavelength selection	Method-dependent, freely selectable
Spectral bandwidth	≤ 4 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 Additional 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
Overall cuvette height	Min. 36 mm
Height of the light beam in the cuvette	8.5 mm
Keyboard	22 foil keys 6 foil keys as softkeys
Result output	Absorbance, transmission, concentration, restricted scan (absorbance wavelength spectrum) Additional, method-dependent data (ratio, background absorbances)
Display	VGA TFT display, 5.7"
Operator guidance language	English, French, Spanish, Italian, German, Japanese
Interfaces	USB master: for USB stick and DPU-S445 thermal printer USB slave: for connecting to a PC RS 232 serial port: for DPU-414 thermal printer RJ45 Ethernet interface: for connecting to a network Connected devices must meet the safety requirements specified in IEC 60950-1.

**Technical data**

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**11.6 Application parameters**

Methods	<p>Preprogrammed and freely programmable methods for all measuring and evaluation procedures:</p> <ul style="list-style-type: none"> <li>• Absorbance measurements</li> <li>• Transmission measurement on a wavelength</li> <li>• Nucleic acids and proteins, OD600</li> <li>• Methods with evaluation via factor, standard and standard series</li> </ul>
Method-dependent evaluation	<p>Absorbance, concentration via factor and standard. Concentration via standard series:</p> <ul style="list-style-type: none"> <li>• Linear regression</li> <li>• Nonlinear regression (2nd and 3rd degree polynoms)</li> <li>• Spline evaluation</li> <li>• Linear interpolation (point-to-point evaluation)</li> </ul> <p>Additional data for nucleic acids: ratios 260/280 and 260/230; molar concentration, total yield</p>
Method memory	>100 method programs
Measured value memory and calibration memory	<p>Memory for &gt;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.)</p>

## 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.

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**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.
- Calculation of ratios 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 Transmission**

In the **Absorbance** method group it is possible to determine the transmission in percent (T%) in addition to the absolute absorbance.

$$T [\%] = 10^{-A} \times 100$$

A = absorbance

T = transmission

### 12.3 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  $A_S$ .

## 12.4 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 has 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.

## 12.5 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

## 12.6 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.

### 12.6.1 Ratios A260/A280 and A260/A230

Application : Information on the purity of the measured nucleic acid. The evaluation of the ratios **A260/A280** and **A260/A230** is activated in the method 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).

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**12.6.2 Conversion to molar concentrations and nucleic acid quantities**

The conversion only can be applied to nucleic acids. It is realized in the **process results/More calculations** method step.

**12.6.2.1 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:  $\mu\text{g}$ .

$C$  = nucleic acid concentration calculated from the measurement. Unit:  $\mu\text{g/mL}$  or  $\text{ng}/\mu\text{L}$ .

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

**12.6.2.2 Calculation of the molar concentration**

Application : Calculating 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:  $\text{pmol/mL}$ .

$C$  = nucleic acid concentration calculated from the measurement. Unit:  $\mu\text{g/mL}$  or  $\text{ng}/\mu\text{L}$ .

$MM$  = relative molar mass. Unit:  $\text{kDa}$

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:  $\text{kDa}$

$bp$  = 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.

### 12.6.3 Calculating the factor for protein in "General Method Parameter"

This section only covers the calculation of the protein component in the **Proteins direct UV** method group. For this method group, the protein component is selected in the parameters (see *Method parameters on p. 32*). 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:

$$A_{0.1\%} = \frac{\epsilon_p}{MM_p}$$

$\epsilon_p$  = molar extinction coefficient of the protein; unit:  $\text{cm}^{-1}\text{M}^{-1}$ .

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

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### 13 Ordering information

Order no. (International)	Order no. (North America)	Description
6133 000.001	–	<b>Eppendorf BioPhotometer D30</b> 230 V/50 – 60 Hz, mains/power plug Europe, more types of mains/power connection available
6133 000.010	6133000010	120 V/50 – 60 Hz, mains/power plug North America
6133 000.907	6133000907	<b>Eppendorf µCuvette G1.0 and BioPhotometer D30 (bundle)</b> Eppendorf microvolume measuring cell and BioPhotometer D30 230 V/50 – 60 Hz
6133 000.908	6133000908	120 V/50 – 60 Hz
6133 928.004	6133928004	<b>BioPhotometer D30 reference filter set</b> Filter set for checking photometric precision and wavelength accuracy (according to NIST)
6135 011.000	6135010004	<b>Thermal Printer DPU-S445</b> including power supply and printer cable
6135 010.004		230 V, EU
6135 012.007		115 V/110V, USA, JP 230 V, UK
0013 021.566	952010409	<b>Thermo paper</b> 5 rolls
6138 000.018	6138000018	<b>Eppendorf µCuvette G1.0</b> Eppendorf microvolume measuring cell for Eppendorf BioPhotometer and BioSpectrometer
0030 106.300	952010051	<b>Eppendorf UVette 220 nm – 1 600 nm</b> Original Eppendorf plastic cuvette, PCR clean, Protein-free 50 - 2 000 µL, 80 pieces, individually packaged
0030 106.318	952010069	<b>Eppendorf UVette routine pack 220 nm – 1 600 nm</b> Eppendorf Quality 50 - 2 000 µL, 200 pieces, reclosable box
0030 079.345	0030079345	<b>Eppendorf macro Vis Cuvettes</b> 10 × 100 pieces
0030 079.353	0030079353	<b>Eppendorf semi-micro Vis Cuvettes</b> 10 × 100 pieces
0030 119.851	0030119851	<b>Eppendorf Cuvette Rack</b> 36 locations, for glass and plastic cuvettes, numbered locations 2 pieces, polypropylene, autoclavable

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# Declaration of Conformity

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

**Product name:**

Eppendorf BioPhotometer® D30

**Product type:**

Photometer

**Relevant directives / standards:**

2014/35/EU: EN 61010-1

UL 61010-1, CAN/CSA C22.2 No. 61010-1

2014/30/EU: EN 55011, EN 61326-1

2011/65/EU: EN 50581

**Date:** December 28, 2015



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