BioPhotometer plus

Operating manual

eppendorf

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Operating manual

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1 User instructions

1.1 Using this manual

- Before using the device for the first time, please read this operating manual.
- Please view this operating manual as part of the product and keep it somewhere easily accessible.
- If this manual is lost, please request another one. The current version can be found on our website, www.eppendorf.com (International) or www.eppendorfna.com (North America).

1.2 Warning signs and hazard icons

Depiction	Meaning
	**DANGER** Risk of electric shock with potential for severe injury or death as a consequence.
	**DANGER** Risk of explosion with potential for severe injury or death as a consequence.
	**DANGER** Biohazard with potential for risk to health or death as a consequence.
	**WARNING** Warning of potential injury or health risk.
	CAUTION Refers to risk of damage to property.
	Refers to particularly useful information and tips.

1.3 Symbols used

Depiction	Meaning
▸	You are requested to perform an action.
1. 2.	Perform these actions in the sequence described.
•	List.
 (example)	Press this key to perform the action described.
Text	Terms used in the device display.

1.4 Abbreviations used

DNA	Deoxyribonucleic acid
dsDNA	double stranded DNA
Dye methods	Group of methods via the keys **dye 550** and **dye 650**
A	Absorbance
FOI	Frequency of Incorporation: measure for the number of dye molecules related to the number of nucleotides in dye-labeled biomolecules
M	mol/l (*molar*)
OD600	Optical density for wave length 600 nm
RNA	Ribonucleic acid
ssDNA	Single stranded DNA
UV	Ultraviolet radiation
VK	Coefficient of variation (standard deviation / mean), in percentages

2 Product description

2.1 Main illustration

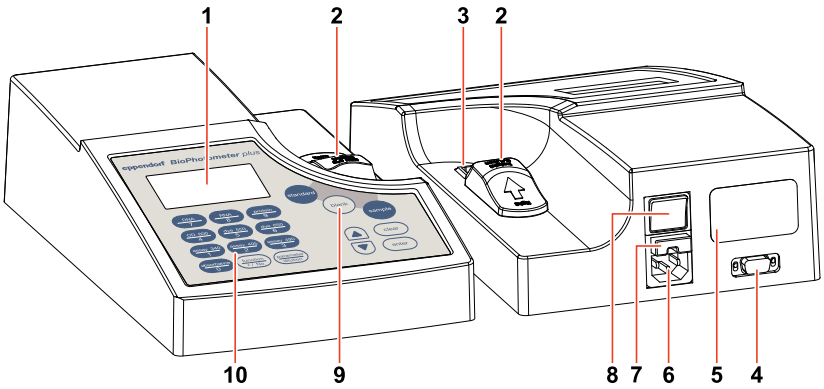

Fig. 1: Front and rear view

1 Device display	**2 Cuvette shaft cover** Slide back or forward to open or close.
3 Cuvette shaft	**4 Connection RS-232**
5 ID plate	**6 Mains connection socket**
7 Fuse holder	**8 Mains switch**
9 Measuring keys	**10 Keyboard**

2.2 Delivery package

Number	Description
1	**BioPhotometer plus**
1	**Power cable**
2	**UVette** Original Eppendorf plastic cuvette, individually wrapped, for direct use in the BioPhotometer, certified RNase, DNA and protein free
1	**BioPhotometer plus operating instructions, multilingual,**

2.3 Features

Cuvette photometer

The BioPhotometer plus is a cuvette photometer for the fast, simple and comfortable measurement of the most important methods in the molecular biology and biochemistry research laboratory. It can also be used for the main photometric methods in cell biology.

Method programs

Method programs for calculating the concentration of nucleic acids, proteins and dye-labeled nucleic acids and proteins as well as the method "OD600" for calculating the bacteria density through measuring turbidity are already preprogrammed. However, you can modify those in many parameters. Other methods for calculating the concentration for 340, 405 and 490 nm can be freely programmed. The method "absorbance" is used for the fast absorbance measurement with any of 9 available wavelengths without further evaluation.

Method programs are combined into groups which you can open quickly via fixed keys.

Cuvettes

You can use standard rectangular glass or plastic cuvettes with optical transparency for the respective measuring wavelength. With the Eppendorf UVette you can also measure nucleic acids and proteins in the UV range using a plastic cuvette. A cover protects the cuvette shaft against dust and other contamination if the photometer is not in use. To open the cuvette shaft it is moved back, to cover it after completing the measurements it is moved forward.

Measuring keys

After opening a method the device is immediately ready for measuring. A measurement is started with one of the 3 round measuring keys.

2 Product description

Evaluation The BioPhotometer plus converts the measured absorbance values into concentration results. Dependent on the method the results can be calculated via fixed factors, standards, or curve calibration. In addition to the results the device also displays the absorbance values and some other important details, e.g. the common absorbance quotients for nucleic acid calculations. Sample dilutions can also be included in the evaluation. Other special evaluation procedures are provided for specific method groups. For example, when calculating the concentration of dyed nucleic acids the frequency of incorporation related to the amount of nucleic acid can also be calculated.

Output The BioPhotometer plus outputs the results via the device display and via a printer available from Eppendorf.

3 Safety

3.1 Intended use

The intended area of use for the BioPhotometer plus is the research laboratory in molecular biology, biochemistry and cell biology. The device may only be operated by trained specialist staff.
The BioPhotometer plus is used to perform photometric measurements to quantify biomolecules as well as to perform turbidity measurements of microbiological cultures in routine laboratories. Due to the specific examination of selected parameters, the device serves to monitor laboratory processes. Use only Eppendorf accessories or accessories recommended by Eppendorf AG.

3.2 Warnings for intended use

Danger! Electric shock from damage to device/power cable.

- Only switch on the device if the device and the power cable are undamaged.
- Only use devices that have been properly installed or repaired.

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

- Switch off the device and disconnect it from the power supply before starting cleaning or disinfecting.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not disinfect by means of spraying.
- Only reconnect the device to the power supply once it is completely dry.

Danger! Electric shock.

- Switch off the device and disconnect the power plug before opening the device to replace the fuses. These tasks may only be performed by appropriately trained staff.

Risk of explosion!

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

Risk when handling toxic or radioactively-marked liquids or pathogenic germs.

- Follow national regulations governing the handling of these substances.
- For complete instructions regarding the handling of germs or biological material of risk group II or higher, please refer to the "Laboratory Biosafety Manual" (Source: World Health Organization, current edition of the Laboratory Biosafety Manual).

Warning! Damage to health from chemicals.

Hazardous chemicals cause burns and other health hazards.

- Follow the instructions for use provided by the manufacturers of reagents and other chemicals.

Warning! Poor safety due to incorrect accessories.

The use of accessories and spare parts other than those recommended by Eppendorf may impair the safety, function and precision of the device. Eppendorf accepts no warranty or liability for damage caused by third-party parts or incorrect use.

- Use only original accessories recommended by Eppendorf.

3 Safety

Warning! Risk to health from contaminated device

- Perform decontamination before storing or dispatching the device and/or its accessories.

Caution when using aggressive chemicals.

Aggressive chemicals may damage both the device and its accessories.

- Do not use any aggressive chemicals on the device or its accessories, such as strong and weak alkalis, strong and weak acids, acetone, formaldehyde, chlorinated hydrocarbons or phenol.
- If the device becomes contaminated with aggressive chemicals, clean it immediately with a neutral cleaning agent.

Caution! Corrosion from aggressive cleaning agents and disinfectants.

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

Caution! Damage to electronic components from condensation.

- After moving the device from a cooler environment (e.g., cool room or outdoors), wait at least an hour before connecting it to the mains power supply.

Caution! Function may be impaired by mechanical damage.

- After a mechancial damage to the device ensure by means of an inspection that the measuring and evaluation functions of the device function correctly.

Caution! Damage due to overheating.

- Do not place the device close to sources of heat (e.g., radiator, drying cabinet).
- Do not expose the device to direct sunlight.
- Allow air to circulate freely by leaving at least 5 cm to adjoining devices or to the wall and keep the underside of the device free.

Caution! 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 cases of doubt, contact the product manufacturer.

Caution! Poor safety due to missing operating manual.

- When passing on the device, always enclose the operating manual.
- If you lose the operating manual, request a replacement. The current version of the operationg manual and the safety instructions can also be found on our website www.eppendorf.com.

Caution! Damage as a result of incorrect packing.

Eppendorf accepts no warranty or liability for damage caused by incorrect packing.

- Only dispatch the device in the original packaging provided for carriage.

3 Safety

Caution! Damage from improper cleaning of the cuvette shaft.

- Only clean the cuvette shaft using a moist cotton swab.(see *Cleaning* on page 32)
- Do not allow any liquid to enter the cuvette shaft.
- Do not reach with your fingers into the cuvette shaft.

Caution! Faulty measurement due to device confusion.

- If you use the devices Biophotometer 6131 and BioPhotometer plus 6132 in your laboratory, note the different method designations on the keys.

3.3 Application limits

Risk of explosion!

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

3.4 Note on product liability

In the following cases, the protection provided in the device may be impaired: Liability for the function of the device passes to the operator if:

- the device is not used in accordance with the operating manual.
- the device is used outside the sphere of application described here.
- the device is used with accessories and consumables (e.g. tubes and plates), which are not recommended by Eppendorf AG.
- the device is maintained or repaired by persons not authorized by Eppendorf.
- the owner has made unauthorized modifications to the device.

4 Installation

4.1 Preparing installation

- Keep the transport carton and the packing material for subsequent safe transport or storage.
- Check the completeness of delivery based on the details of the scope of delivery (see *Delivery package* on page 8).
- Check all parts for any transport damage.

4.2 Selecting location

Select the location for the BioPhotometer plus in accordance with the following criteria:

- 2 power sockets with ground conductor for the BioPhotometer plus and the printer.
- Solid laboratory bench with horizontal work surface
  Space requirement of the device: 40 cm (with printer: 65 cm) width, 50 cm depth.
- Temperature: 15 to 35 °C. Avoid direct sunlight.
- Humidity: 25 to 75 % relative humidity.
- Atmospheric pressure: 70 to 106 kPa.

4.3 Connect device to the main power supply

1. Place the BioPhotometer plus onto a suitable work surface.
2. Ensure that the mains voltage and frequency match the details for the range of mains voltages and frequencies on the device nameplate.
3. Connect the device to the power supply and switch it on from the mains power switch **8** (Fig. 1 on p. 8).
4. Remove the protective film from the device display.

4.4 Cuvettes

You can insert standard rectangular glass or plastic cuvettes into the cuvette shaft (outside diameter 12.5 mm x 12.5 mm). The optical path height must be 8.5 mm above the cuvette base and the total cuvette height must be at least 36 mm. The light beam in the cuvette is 1.0 mm wide and 1.5 mm high.

The cuvettes must be optically transparent for the respective measuring wavelength. For measurements in the UV range Eppendorf provides a plastic cuvette called UVette which is transparent from wavelengths above 220 nm and therefore also suitable for the measurement of nucleic acids.

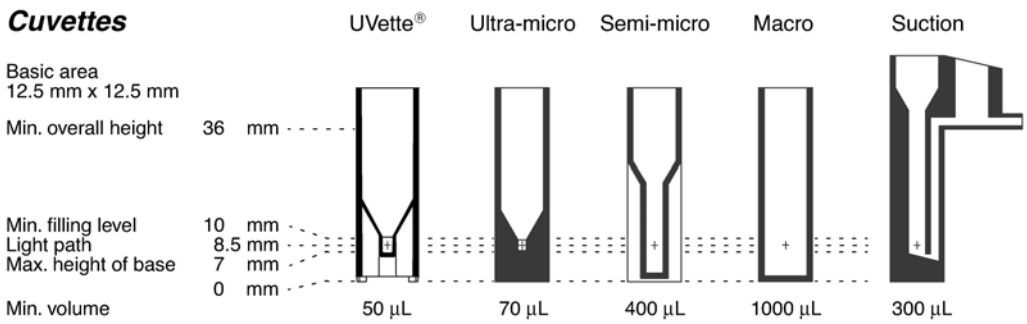

Fig. 2: Overview of different cuvette types

4 Installation

4.5 Connect printer

You can connect the Eppendorf thermal printer to the serial interface RS-232 C of the photometer (see *Ordering information* on page 48).

1. Connect the printer cable to the serial printer port **4** of the photometer and tighten the locking screws.
2. Connect the printer cable to the printer and also tighten the locking screws.
3. Connect the printer to the power supply using the plug-in power unit supplied (printer accessory) and switch it on.
4. Check the printer settings in accordance with the following table and make corrections where necessary.

   Information about modifying printer settings can be found in the operating manual for the printer.

Tab. 1: Setting the DIP SW for the thermal printer

DIP SW-1	Meaning
1 (OFF)	Input = Serial
2 (ON)	Printing Speed = High
3 (ON)	Auto Loading = ON
4 (OFF)	Auto LF = OFF
5 (ON)	Setting Command = Enable
6 (OFF)	Printing
7 (ON)	Density
8 (ON)	= 100%

DIP SW-2	Meaning
1 (ON)	Printing Columns = 40
2 (ON)	User Font Back-up = ON
3 (ON)	Character Select = Normal
4 (ON)	Zero = Normal
5 (ON)	International
6 (ON)	Character
7 (ON)	Set
8 (OFF)	= U.S.A.

DIP SW-3	Meaning
1 (ON)	Data Length = 8 bits
2 (ON)	Parity Setting = NO
3 (ON)	Parity Condition = Odd
4 (OFF)	Busy Control = XON/XOFF
5 (OFF)	Baud
6 (ON)	Rate
7 (ON)	Select
8 (ON)	= 9600 bps

5 Operation

5.1 Overview of operating controls

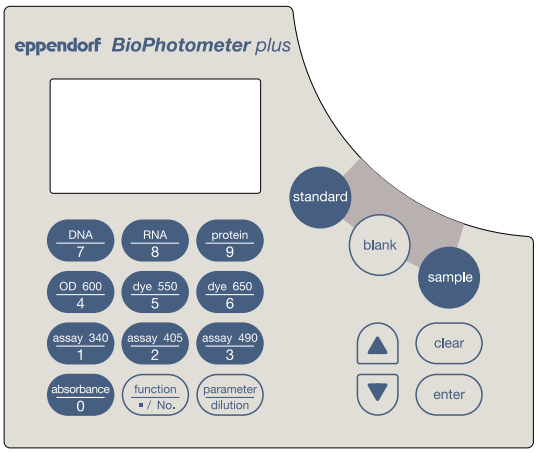

Fig. 3: Control panel of the BioPhotometer plus.

Key	Function
Oval blue keys, e.g.:	
DNA 7	• In the method selection: select method group. • When entering values: enter digits.
Circular keys	
standard	Start standard measurement.
blank	Start blank measurement.
sample	Start sample measurement.
Oval transparent keys	
parameter dilution	• In the method selection: open parameter list. • During the measurement procedure: enter sample dilution.
function ▪ / No.	• In the method selection: open function list. • During the measuring procedure: modify sample number. • When entering digits: enter decimal point.
enter	• Confirm entry or selection. • Open selected method or function.
clear	Delete entry
Cursor keys	
▲ ▼	Move cursor up or down in the device display.

5 Operation

5.2 Methods

The following methods are available and already preprogrammed ex factory. You can modify most parameters and save them as a modified method (see *Parameter* on page 26).

Method group	Method	Explanation	Wavelength
DNA	dsDNA	Calculating the concentration of DNA with evaluation via factor. The methods differ mainly in the preprogrammed factor.	Measuring wavelength: 260 nm Secondary wavelengths to check for purity: 230, 280, 340 nm
	ssDNA		
	OLIGO DNA		
RNA	RNA	Analogous to method group DNA.	As method group DNA.
	OLIGO RNA		
Protein	BCA	Calculating the concentration of proteins after adding reagent. The methods are preprogrammed with the evaluation procedure calibration curve. Number and target concentration of the standards can be modified.	550 nm
	BCA MICRO		
	BRADFORD		595 nm
	BRADFORD MICRO		
	LOWRY		595 nm
	LOWRY MICRO		
	PROTEIN 280 nm	Calculating the concentration of proteins with evaluation via factor.	Measuring wavelength: 280 nm Secondary wavelengths to check for purity: 260, 340 nm
OD600	OD600	Turbidity measurement to determine the bacteria density.	595 nm
dye 550	DYE 550-dsDNA	For dye-labeled biomolecules: calculating the concentration of the molecule (nucleic acid or protein) and the dye in a single measuring procedure. The frequency of incorporation of the dye in the biomolecule is also determined.	DNA/RNA/OLIGO: see method groups DNA and RNA PROTEIN: see method PROTEIN 280 nm Measuring wavelength for the dye: 550 nm
	DYE 550-ssDNA		
	DYE 550-RNA		
	DYE 550-OLIGO		
	DYE 550-PROTEIN		
dye 650	DYE 650-dsDNA	Analogous to method group "dye 550".	DNA/RNA/OLIGO: see method groups DNA and RNA PROTEIN: see method PROTEIN 280 nm Measuring wavelength for the dye: 650 nm
	DYE 650-ssDNA		
	DYE 650-RNA		
	DYE 650-OLIGO		
	DYE 650-PROTEIN		
assay 340	ASSAY 340/1	Calculating the concentration by measuring at 340 nm. The evaluation procedures can be freely programmed. As a sample the methods are already preprogrammed with the following evaluation procedures: 340/1: evaluation via factor 340/2: evaluation via a standard. 340/3: evaluation via calibration curve with 6 standards.	340 nm
	ASSAY 340/2		
	ASSAY 340/3		
assay 405	ASSAY 405/1	Analogous to method group "assay 340".	405 nm
	ASSAY 405/2		
	ASSAY 405/3		
assay 490	ASSAY 490/1	Analogous to method group "assay 340".	490 nm
	ASSAY 490/2		
	ASSAY 490/3		
ABSORBANCE	ABSORBANCE	Rapid absorbance measurement after selecting the wavelength.	230, 260, 280, 340, 405, 490, 550, 595, 650 nm

5 Operation

5.3 Summary of the measuring procedure

This section contains a summary of the key steps of a measuring procedure.

5.3.1 Prepare measurement

1. Switch on the device and, if necessary, the printer also.
   The device is immediately ready for measuring after being switched on.
2. Have the cuvettes for the measurements available. When selecting the cuvettes observe the respective instructions (see *Cuvettes* on page 13).
3. Provide the measuring solutions for measuring the blanks and, if necessary, the standards and samples.
   Measuring solutions for standards and samples with lower absorbances than 0.02 to 0.03 A (this range corresponds e.g. to a dsDNA concentration of 1.0 to 1.5 µg/ml) should not be used. Whilst the detection level of the photometer is well below these values, the effect of interferences from the measuring solutions (particles, bubbles, turbidity) on the reliability of the results is very high for these low absorbances.
4. Slide the cover of the cuvette shaft back to open the cuvette shaft.

5.3.2 Select the method

1. Using a key select the method group.
   In the device display you see a list of methods provided in the selected group.
2. Using the cursor keys select the desired method.

Before opening the method you should check the parameters of the desired method and correct them, if necessary (see *Parameter* on page 26).

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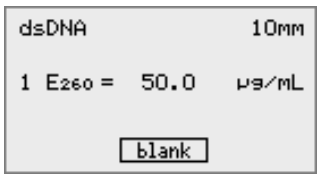
3. Open the selected method using the **Enter** key.
   The startup screen of the method is displayed:



- Top: method name and programmed optical path length of the cuvette.
- Center: programmed evaluation (e.g. factor or information about the evaluation with standards).
- Bottom: keys for the next measurement. The keys are not shown if there is insufficient space to display them, but they can be made visible using the **Enter** key in this case.

The displayed factor defined in the method parameters always relates to an optical path length of 10 mm. However, for calculating the result the device automatically takes into account the optical path length defined in the method parameters. Therefore, you do not need to modify the factor in the method parameters for other optical path lengths.

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5 Operation

5.3.3 Measure

Check for each measurement:

- Is there enough measuring solution in the cuvette? The light path height of the BioPhotometer plus is 8.5 mm. The height of the light beam in the cuvette is 1.5 mm.
- Is the measuring solution free from particles and bubbles?
- Is the measuring surface of the cuvette free from contamination due to dust or finger prints and free from scratches?
- When inserting the cuvette press it all the way down against a slight resistance.
- Is the cuvette positioned correctly? The optical surface of the cuvette must point towards the direction of the light path. The direction of the light path in the BioPhotometer plus is indicated by an arrow on the blue cuvette shaft cover.
- For plastic cuvettes: How many consecutive measurements can be reliably carried out in the cuvette?
- Carry out a blank measurement for each cuvette before any sample or standard measurement to compensate for the cuvette blank in addition to the reagent blank value.
- Check whether the measured absorbance values exceed the upper limit of the photometric measuring range. Discard the measuring result in this case. The upper limit of the photometric measuring range not only depends on the wavelength (see *Photometer* on page 41) but also on the cuvette blank. Ultramicro cuvettes with a small aperture such as "TrayCell" (Hellma) or "LabelGuard" (Implen) may have a cuvette blank of up to A = 1. The available photometric measuring range is reduced by this amount. You can estimate the cuvette blank if you measure a water-filled cuvette as a sample against the empty cuvette shaft as a blank.
- Remove the measuring solution completely after measurement before filling the next measuring solution in order to minimise carry-over. If a carry-over between samples is expected due to the high concentration differences then flush the cuvette between measurements.
- With temperature differences between the lamp and the environment photometric drift may occur. Therefore a device being brought in from a colder environment should first reach the ambient temperature. Alternatively you can bring the lamp to the right temperature by carrying out a few measurements. In long series of measurements or in measurements over a long period of time carry out a new blank measurement.

blank

1. Open the cuvette shaft by sliding the blue cover back.
2. Fill the cuvette with blank solution and insert the filled cuvette into the cuvette shaft.
3. Press the **blank** key.

- Top: method name and display of the sample type (here: "BLANK")
- Center: result (for blank: 0.000 A)

Blank results remain stored as long as the method remains open. However, we recommend to check the blank at regular intervals of e.g. one hour. To do so carry out a measurement with the blank solution as sample. If the measuring result differs significantly from 0 a new blank measurement must be carried out.

Standards (optional)

4. Only for methods with standard evaluation: Measure the required standards consecutively if you want to carry out a new calibration (see *Methods with evaluation via standards* on page 21).

Samples

5. Fill the cuvette with sample solution and press the **sample** key.

5 Operation

5.3.4 Finalize the method

1. Press one of the method group keys to return to the method selection and open the next method, if necessary.
2. After all measurements are complete switch off the device.
3. Slide the cuvette shaft cover forward to protect the shaft against contamination.

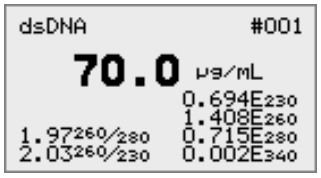
5.4 Nucleic acids

The methods provided in the method groups "DNA" and "RNA" differ mainly in the preprogrammed factor.

Additionally, for the methods "OLIGO DNA" and "OLIGO RNA" the selection of the parameter "MOL. UNIT" (molar concentration unit) differs from that of other nucleic acid methods. This parameter is only required for special conversions described at the end of this section.

As optical path length of the cuvette the value "10 mm" has been preprogrammed. If you modify the value the modified optical path length is taken into account by the device when calculating the results. Therefore you do not need to change the factor for the evaluation.

Results display

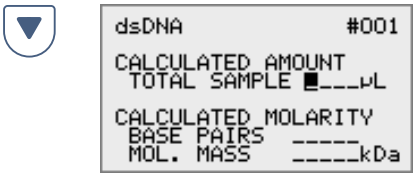


Top: method name and sample number
Center: result and unit
Bottom left: absorbance quotient ("ratios")
Bottom right: absorbance results

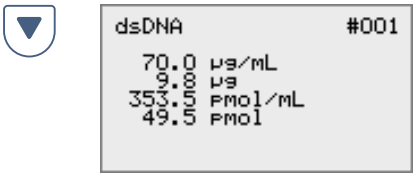
In addition to the concentration result and the absorbance at measuring wavelength 260 nm the absorbances at 3 additional wavelengths and the quotients 260/280 and 260/230 are displayed as an indication for the purity of the measured nucleic acid sample. The absorbance at 340 nm should be near zero for pure samples.

Turbid measuring solutions have raised absorbances at all measuring wavelengths distorting the measuring result. You can partially correct this by enabling the parameter "CORRECTION A_{340}". The absorbance value for 340 nm is then highlighted in the display with the cursor to indicate that the correction is enabled.

The last measured concentration result can be converted into molar concentrations and/ or nucleic acid amounts (mass unit or molar unit) if desired:



Top: input of the total amount of the sample
Bottom: input of the base pairs (for single strand nucleic acid: the bases) or molar mass. One of the two entries suffices.
All entries must be confirmed using the **Enter** key.



Display of the calculated results for the molar concentration and the sample amounts in the total amount.

5 Operation

5.5 Proteins

You can measure the protein concentrations directly by measuring in the UV range at 280 nm or after adding reagent in the VIS range.

5.5.1 Protein 280 nm

The measurements can be evaluated via a factor entered into the parameters or via a single point calibration (measuring a standard).

- **Factor**: This evaluation mode is preprogrammed ex factory. However, you still have to enter the factor before the first measurement.
  If you modify the parameter "CUVETTE" (preprogrammed to 10 mm) the modified thickness of the layer will be taken into account by the device when calculating the results. This means you do not need to adjust the factor for evaluation but have to provide the input for an optical path length of 10 mm.
- **Standard**: Alternatively you can program the evaluation mode "Standard" ("STD") (see *Methods with evaluation via standards* on page 21).

Results display

Top: method name and sample number
Center: result and unit
Bottom right: absorbance results

In addition to the concentration result and the absorbance at measuring wavelength 280 nm the absorbances at 2 additional wavelengths are displayed as an indication for the purity of the measured protein sample. The absorbance at 340 nm should be near zero for pure samples.

Tubid measuring solutions have raised absorbances at all measuring wavelengths distorting the measuring result. You can partially correct the distortion by enabling the parameter "CORRECTION A_{340}".

5.5.2 Protein after adding reagent (Bradford, BCA, Lowry)

You can evaluate these methods via factor or calibration (standard measurement).

- **Factor**: If you evaluate via factor you must take into account when making the entry that the factor is adjusted to the selected result unit.
  If you modify the parameter "CUVETTE" the modified optical path length is, however, taken into account by the device when calculating the results. This means you do not need to adjust the factor for evaluation but have to provide the input for an optical path length of 10 mm.
- **Standard**: For evaluation via calibration you can program up to 10 different standards for these methods. The procedure for calibration is described in the next chapter (see *Methods with evaluation via standards* on page 21).

5 Operation

5.6 Methods with evaluation via standards

For the following methods you can define an evaluation via calibration (measuring of standards) in the parameters:

- PROTEIN 280nm (here only calibration with one standard is possible)
- BRADFORD, BRADFORD micro, BCA, BCA micro, LOWRY, LOWRY micro
- Methods via the keys "assays 340 / 405 / 490"

You can program up to 10 standards each in single up to triple measurement. For evaluation with several standards you can choose between the procedures "Linear regression" (for calibration lines) and "Non-linear regression" (for calibration curves). Dependent on the number of programmed standards the following applies:

Evaluation procedure	Number of standards
Calculation of a factor	1
Linear regression	2 to 10 standards
Non-linear regression	for single measurement: 5 to 10 standards for double or triple measurement: 4 to 10 standards

Measuring procedure

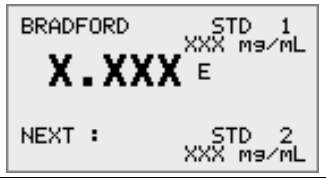
Standard measurements remain stored for an unlimited period of time after a valid calibration until they are overwritten with a new calibration. Exception: If method parameters are modified the calibration is deleted.

After opening a method (in the example the method "BRADFORD") you see the following device display:

The values marked "XXX" depend on the standard concentrations which you have programmed in the method parameters.

Because a calibration has already been stored in this example, the measuring keys provided include "sample" in addition to "standard" and "blank".
Carry out a blank measurement.

Measure the first standard (in this example only as single measurement).



Measure the other standards as prompted in the bottom area of the device display.

After completing all standard measurements the calibration has been evaluated and stored.
When measuring more than 2 standard samples a CV value is displayed for the calculated regression. If the calculated CV value exceeds 10% you are prompted for approval before saving. Sample measurements are evaluated using the last valid calibration.

After saving the calibration you can continue with a sample measurement.

In the function list (see *Functions* on page 31) you can view and print the stored calibration data.

5 Operation

5.7 OD 600

With method "OD 600" you can measure the bacteria density via a turbidity measurement at approx. 600 nm. Because this is a stray-light measurement the result depends on the geometry of the light path which can differ for the photometers by different manufacturers.

The exact measurement wavelength for the BioPhotometer plus is 595 nm. Measurements with suspensions of E. coli bacteria (absorbance range: approx. 0.1 to 0.3 E) showed at 600 nm about 1 to 2 % higher absorbance values than at 595 nm. You can program a corresponding correction factor in the parameters.

Results display

Top: method name and sample number
Center: result.
Bottom right: measured absorbance.

5.8 Dye-labeled biomolecules ("dye methods")

The preprogrammed methods for the keys **dye 550** and **dye 650** contain measuring procedures for dye-labeled biomolecules. During these measuring procedures both the biomolecule (nucleic acid or protein) and the dye are measured at different wavelenghts and their concentrations are determined. In addition the frequency of incorporation (concentration ratio between dye and biomolecule) is calculated.

5.8.1 Method group "dye 550"

The dye is measured at 550 nm, the nucleic acid at 260 nm and the proteins at 280 nm (see *Methods* on page 16). A selection of 4 dyes is available in the method parameters:

- CY 3
- ALEXA 546
- ALEXA 555
- DYE 550

The first 3 dyes are used more frequently in the laboratory, the last dye (DYE 550) is a spaceholder for other dyes.

For every dye you have to program the following corresponding data in the parameters. For the Cy and Alexa dyes values have already been preprogrammed but can be modified:

- Absorbance coefficient in the unit $cm^{-1} \cdot M^{-1}$. The device uses this to calculate the factor for converting the absorbance into concentration related to a cuvette optical path length of 10 mm and displays this on the start-up screen after opening the method.
- Optional: factors for the correction calculation "CORR: A_{550}" (see below for explanation).

The parameters for the nucleic acid or protein component mainly match the parameters of the method group *DNA* and *RNA* and for the method *Protein 280 nm*.

5.8.2 Method group "dye 650"

The dye is measured at 650 nm. A selection of 3 dyes is available:

- CY 5
- ALEXA 647
- DYE 650

The additional explanations found under *method group "dye 550"* apply accordingly.

5 Operation

5.8.3 Frequency of incorporation "FOI"

The FOI is a measure for the concentration of the dye relative to the concentration of the nucleic acid. It is not provided for protein methods. For calculating the FOI you can select between 2 units in the method parameters:

- "MOLECULE DYE / kb": dye molecules per 1000 nucleotides.
- "pmole/µg DNA": pmole dye per µg nucleic acid.

The formulae for calculating the FOI can be found separately (see *Calculation of the FOI* on page 45).

5.8.4 Correction factors

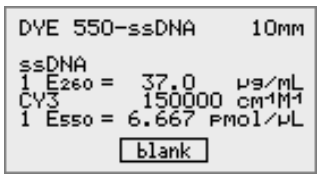
In addition to the options for turbidity correction described (parameter "CORRECTION A_{340}") (see *Nucleic acids* on page 19) the affect of the dye on the measurement of the nucleic acid or the protein can also be corrected for the "DYE methods". If the degree to which the dye absorbs light is known also for the measuring wavelengths of the biomolecule (260 and 280 nm), then a correction can be made in the parameter "CORRECTION$_{550\ (or\ 650)}$". If you enable this parameter you can enter a correction factor for 260 and 280 nm. E.g. for Cy 3 the preprogrammed values are:

- 0.04 for "CORR.A_{550}: F_{260}"
- 0.05 for "CORR.A_{550}: F_{260}"

The use of these factors for the calculation of concentration and ratio of the biomolecule is described elsewhere (see *Correction $A_{550/650}$* on page 46).

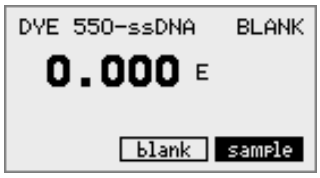
5.8.5 Measuring procedure and result display

After calling the method you will see the following display (example: "DYE 550 - ssDNA"):

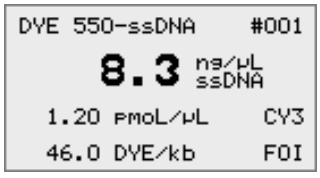


Top: method name and cuvette selected in the parameters.
Center: programmed values for the calculation of the DNA and dye concentrations: factor for ssDNA and absorbance coefficient for the dye. In addition the factor calculated from the absorbance coefficient related to a cuvette optical path length of 10 mm will be displayed.
Bottom: measuring key for the next measurement.

▸ Carry out a blank measurement.



▸ Carry out a sample measurement.



Top: method name and sample number
Center: result for DNA and dye.
Bottom: result for the frequency of incorporation.

5 Operation

As with the nucleic acid measurement without dye measurement (see *Nucleic acids* on page 19) you can use the cursor keys to access additional details of the result (absorbance value and ratio 260/280) and to carry out conversions for further results:

After entering the total volume of a sample:	Total amount of the nucleic acid (mass in µg) and the dye (in pmole).
After entering the bases or molecular mass:	Molar concentration of the nucleic acid. If the total amount of the sample has also been entered: total amount of the nucleic acid (in pmole).

When displaying the absorbance values the values for 340 nm or for 550 (or 650) nm are highlighted with the cursor if "CORRECTION A_{340}" or "CORRECTION $A_{550\ (or\ 650)}$" has been enabled.

5.9 Methods for 340, 405 and 490 nm

3 method locations are provided for each wavelength. The following evaluation procedures have been preprogrammed as samples. However, you can adjust these procedures at any time:

Example: "assay 340"

- ASSAY 340/1: Evaluation via factor
- ASSAY 340/2: Evaluation via single point calibration
- ASSAY 340/3: Evaluation via curve calibration

The following applies to evaluation via factor:

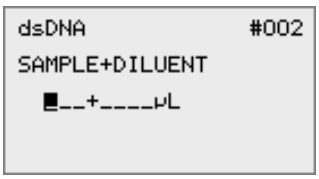
- If you modify the parameter "CUVETTE" (preprogrammed to 10 mm) the modified thickness of the layer will be taken into account by the device when calculating the results. This means you do not need to adjust the factor for evaluation but have to provide the input for an optical path length of 10 mm.
- When entering the factor ensure that it has been adjusted for the selected unit of results.

5.10 Dilution

You can enter sample dilutions before a measurement using the **parameter/dilution** key. The dilution factor will then be automatically taken into account when calculating the results.

For the following example the method has already been called and a blank been measured:

1. Open the dilution input using the **parameter/dilution** key.

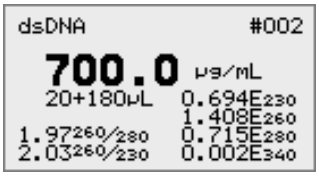


2. Enter the volumes of the sample and the dilution buffer ("diluent") and confirm each entry with **enter**.

   This brings you back to the measuring procedure and you can start a sample measurement.

5 Operation

3. Start a sample measurement using the **sample** key.



4. The result is calculated taking into account the sample dilution entered.

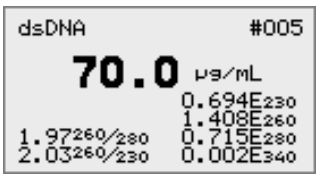
The dilution is retained for calculating all subsequent sample results until it is overwritten. To delete the dilution enter "0" for both "SAMPLE" and "DILUENT" or delete the values using the keys **clear** and **enter**.

5.11 Sample number

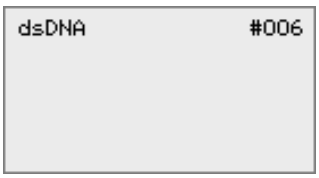
The device display for measuring results contains the sample number at the top right. It is counted separately continuously for every method and reset to "1" when a new method is opened.

You can modify the sample number before a measurement.

For the following example 5 samples were already measured:



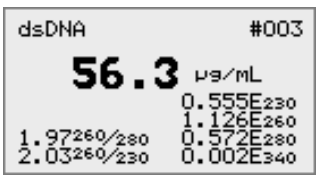
1. Open the sample number input using the **function**/-/**No.** key.



2. Enter the desired sample number for the next sample (here "3") and confirm the entry with **Enter**.

   This brings you back to the measuring procedure and you can start a sample measurement.

3. Start a sample measurement using the **sample** key.



4. The sample result is allocated the sample number "3". All other samples are incremented continuously.

6 Parameter and functions

6.1 Parameter

The methods of the BioPhotometer plus as well as the corresponding parameters have already been preprogrammed ex factory. You can modify these parameters.

6.1.1 View, change and store the parameters of a method

1. Select the desired method group using the corresponding method key.
2. Using the cursor keys select the desired method.
3. Using **parameter/dilution** open the parameters for this method.
4. Scroll through the parameter list with the cursor keys.
5. Modify the parameters, if necessary, and confirm the changes using **Enter**.
   There are two kinds of parameter entries:
   - Selection parameters: selection via cursor keys.
   - Numerical values: input via number keys.

Each parameter entered is only saved after confirmation using the **Enter** key.

6. Exit the parameter list by pressing **parameter/dilution** again.
   You return to the method selection.

6.1.2 Summary and description of parameters

Parameter	Entry	Explanation
Cuvette	Selection: 10, 5, 2, 1 or 0.2 mm	Optical optical path length of the cuvette. The factor for converting the absorbance into the concentration is corrected accordingly internally.
Unit	Selection: Different units dependent on the method.	Result unit for the method. In some methods this selection is missing because a unit has been preprogrammed permanently.
MOL. Unit	Selection: Different units dependent on the method.	Molar unit, only for the nucleic acid methods: for the conversion of concentration units into molar concentrations.
CALCULATION	Selection: • STD • FACTOR	Evaluation procedure for calculating the sample concentration: • with fixed factor • with standards (calibration)
Factor	Numerical input (5 digits)	Factor for converting absorbance into concentration. The number of digits after the decimal point of the factor determines the number of digits after the decimal point for the result. The factor must be entered for a cuvette optical path length of 10 mm.
STD NUMBER 1-10	Numerical input (1 to 10)	Number of standards with different concentrations used for the calibration.
STD MEASUREMENT	Selection: 1x, 2x or 3x	Repeated measurements of standards.

6 Parameter and functions

Parameter	Entry	Explanation
REGRESSION	Selection: Linear and non-linear regression	Evaluation procedure of the multi-point calibration. • Linear regression is possible for: 2 to 10 standards • Non-linear regression is possible for: (single measurement:) 5 to 10 standards (double or triple measurement:) 4 to 10 standards.
STD 1 to STD 10	Numerical input (numbers and decimal point, 5 digits)	Nominal concentration for each standard. Unit as entered in the parameter display 1. The number of digits after the decimal point in the nominal concentration of the first standard determines the number of digits after the decimal point in the result. The concentrations must be entered in ascending order.
CORRECTION	Selection: • NO CORRECTION • CORRECTION A_{340} • CORRECTION $A_{550\ (or\ 650)}$	CORRECTION A_{340} Can be used for the partial correction of the effect of minor turbidity in the measuring solution: The absorbance measured at 340 nm is subtracted from the absorbance results at 230, 260 and 280 nm prior to subsequent evaluation. CORRECTION $A_{550\ (or\ 650)}$ Can be used for the "Dye methods" to correct the effect of the dye on the measuring absorbances of the biomolecule (nucleic acid or protein): The absorbance of the dye measured at 550 (or 650) nm is multiplied with a correction factor for 260 or 280 nm. The result is subtracted from the absorbances of the biomolecule at 260 or 280 nm prior to subsequent evaluation for the biomolecule (see *Dye-labeled biomolecules ("dye methods")* on page 22). The correction factors are entered further down in the parameter list after selecting the dye.
CY3 (or Cy5) ALEXA	Selection	For "Dye methods": selection of the dye (see *Dye-labeled biomolecules ("dye methods")* on page 22).
ABS.COEFF.	Numerical input (6 digits)	For "Dye methods": input of the absorbance coefficient for the selected dye in the unit "$cm^{-1} \cdot M^{-1}$".
CORRECTION $A_{550\ (650)}$: F_{260} CORRECTION $A_{550\ (650)}$: F_{260}	Numerical input (5 digits)	Only if in "Dye methods" the parameter CORRECTION $A_{550\ (or\ 650)}$ has been enabled: Entry of the correction factors for taking into account the effect of the dye on the measurement absorbances of the biomolecule (see description of the parameter CORRECTION $A_{550\ (or\ 650)}$ above).
CALCULATION FOI	Selection: • MOLECULE DYE / kb • pmole/µg DNA (or RNA)	For "Dye methods": selection of the procedure for calculating the frequency of incorporation (see *Dye-labeled biomolecules ("dye methods")* on page 22).

6 Parameter and functions

6.1.3 Parameters preprogrammed ex factory

Nucleic acids and OD 600

Parameter	dsDNA	ssDNA	OLIGO DNA	RNA	OLIGO RNA	OD 600
CUVETTE	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm
UNIT	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	
MOL. UNIT	pmol/mL	pmol/mL	pmol/µL	pmol/mL	pmol/µL	
FACTOR	50.0	37.0	30.0	40.0	30.0	1.000
CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION	

Proteins

Parameter	BRADFORD	BRADFORD micro	BCA	BCA micro	LOWRY	LOWRY micro	PROTEIN 280 nm
CUVETTE	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm
UNIT	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	mg/ml (no entry, programmed permanently)
CORRECTION							NO COR-RECTION
CALCULATION	STD	STD	STD	STD	STD	STD	Factor
FACTOR							-----
STD NUMBER 1-10	6	6	8	5	6	6	
STD MEASUREMENT	1x	1x	1x	1x	1x	1x	
REGRESSION	NON-LINEAR	NON-LINEAR	NON-LINEAR	NON-LINEAR	NON-LINEAR	NON-LINEAR	
STD 1	100	1.00	25	0.50	100	1.00	
STD 2	250	2.5	125	2	250	2.5	
STD 3	500	5	250	5	500	5	
STD 4	750	10	500	10	750	10	
STD 5	1000	15	750	20	1000	15	
STD 6	1500	25	1000		1500	25	
STD 7			1500				
STD 8			2000				

6 Parameter and functions

Method group "dye 550"

Parameter	DYE 550-dsDNA	DYE 550-ssDNA	DYE 550-RNA	DYE 550-OLIGO	DYE 550-PROTEIN
CUVETTE	10 mm	10 mm	10 mm	10 mm	10 mm
Unit (only for nucleic acid)	µg/mL	µg/mL	µg/mL	µg/mL	mg/mL (protein: no entry, programmed permanently)
MOL. UNIT (for nucleic acid)	pmol/mL	pmol/mL	pmol/mL	pmol/µL	
FACTOR (for nucleic acid or protein)	50.0	37.0	40.0	30.0	-----
CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION	NO CORRECTION
Dye selection	CY3	CY3	CY3	CY3	CY3
ABS.COEFF.	(for Cy3:) 150000	(for Cy3:) 150000	(for Cy3:) 150000	(for Cy3:) 150000	(for Cy3:) 150000
CALCULATION FOI	MOLECULES DYE / kb	MOLECULES DYE / kb	MOLECULES DYE / kb	MOLECULES DYE / kb	

The unit for the dye is defined permanently with "pmol/µl" and can therefore not be programmed in the parameters.

For the various dyes the following dye-specific values have been preprogrammed as absorbance coefficients and correction factors (for the parameter CORRECTION A_{550}):

Parameter	CY3	ALEXA546	ALEXA555	DYE550
ABS.COEFF.	150000	------	150000	------
CORRECTION A_{550}: F_{260}	0.04	0.0	0.04	0.0
CORRECTION A_{550}: F_{280}	0.05	0.0	0.04	0.0

The values for ALEXA546 were not known at the time of writing this manual; please contact the manufacturer (Invitrogen) with regard to these values.

Method group "dye 650"

The parameters correspond mainly to those of method group "dye 550". As preselection for the dye Cy 5 has been programmed. For the dye-specific values the following applies:

Parameter	CY5	ALEXA647	DYE650
ABS.COEFF.	250000	239000	------
CORRECTION A_{650}: F_{260}	0.0	0.0	0.0
CORRECTION A_{650}: F_{280}	0.05	0.03	0.0

6 Parameter and functions

assay 340

Parameter	ASSAY 340/1	ASSAY 340/2	ASSAY 340/3
CUVETTE	10 mm	10 mm	10 mm
UNIT	µg/mL	µg/mL	µg/mL
CALCULATION	Factor	STD	STD
FACTOR	-----		
STD NUMBER 1-10		1	6
STD MEASUREMENT		2x	1x
REGRESSION			NON-LINEAR
STD 1		-----	-----
STD 2			-----
STD 3			-----
STD 4			-----
STD 5			-----
STD 6			-----

assay 405 and assay 490

The parameters correspond to those of method group "assay340".

ABSORBANCE

You can define the wavelength directly in the start-up screen of the method. If you open the parameter list you are provided with the cuvette optical path length and unit for information only. However, neither parameter can be modified in this method. Unlike with other methods there is no automatic conversion to a different cuvette optical path length in this case.

6 Parameter and functions

6.2 Functions

1. In the method selection press the key **function**/·/**No.**.

   A list of general device functions will open. In the method procedure or parameter mode this key has a different function (entering the sample number or entering a decimal point when inputting values).

2. In this function list and any sub-lists (e.g. results list) select the desired function like in the parameter list (see *View, change and store the parameters of a method* on page 26) using the cursor keys and open it with the **Enter** key.
3. Press the **function**/·/**No.** key again to return from a sublevel of the functions (e.g. opening stored results in the function "RESULTS") to the next higher level in the function list and finally back to the method selection.

Tab. 2: Summary and description of functions

Function	Explanation
RESULTS	Display of the last 100 results (the result measured last is shown first). For results with information about more than one device display (detailed information for nucleic acids and "dye methods") you can access the detailed displays via the cursor keys. Print the selected result using the **Enter** key.
STANDARDS	Display of the stored calibration data for methods with evaluation via standards. In this list you can also scroll using the cursor keys until you see the calibration data of the desired method in the device display.
PRECISION MEASUREMENT	Measurement and precision calculation of 10 consecutive measured values of a sample. The method program of the last opened method is used.
PHOTOMETER TEST NEW MEASUREMENT LAST MEASUREMENT	Checking the photometer using a filter kit from Eppendorf (see *Check photometer* on page 34). You can start a new check (NEW MEASUREMENT) or open the results of the last check (LAST MEASUREMENT).
SPRACHE DEUTSCH LANGUAGE ENGLISH LANGUAGE U.S.ENGL LANGUE FRANCAISE	Selection of the language version. "ENGLISH" and "U.S.ENGLISH" differ in their date format.
DATE	Date input. Save using **Enter**.
Time	Time input. Save using **Enter**.
Printer	Printer selection: • DPU 414: Connection of the Eppendorf thermal printer. • SERIAL: Connection of a different printer. In this case certain printer requirements must be met. Contact your Eppendorf partner for details.
SERVICE	The function is only available to Eppendorf Service.

7 Maintenance

7.1 Cleaning

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

- Switch off the device and disconnect it from the power supply before starting cleaning or disinfecting.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not disinfect by means of spraying.
- Only reconnect the device to the power supply once it is completely dry.

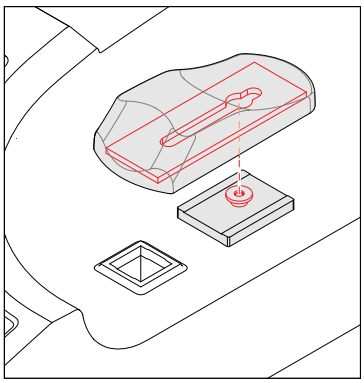
Caution! Corrosion from aggressive cleaning agents and disinfectants.

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

1. Switch off the device and disconnect the power plug.
2. Wipe down the surfaces with a cloth you have moistened with a mild cleaning agent.
3. Only clean the cuvette shaft using a moist lint-free cotton swab. Prevent liquid from entering the cuvette shaft.

7.1.1 Cleaning the cuvette shaft cover

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



1. Slide the cover fully forward.
2. Gently pull the cover upwards near the front and then slowly push it to the back.

   After a few millimeters you can lift the cover completely.
3. Clean the cover and the cover holder with a cloth or a lint-free cotton swab wetted with a mild detergent.
4. Replace the cover on the cover holder as shown here.

   The button of the cover holder fits exactly into the circular enlarged recess at the bottom of the cover.

If the photometer is not in use slide the blue cover over the cuvette shaft to protect it against dust and other contamination.

7 Maintenance

7.2 Disinfection / Decontamination

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

- Switch off the device and disconnect it from the power supply before starting cleaning or disinfecting.
- Do not allow any liquids to penetrate the inside of the housing.
- Do not disinfect by means of spraying.
- Only reconnect the device to the power supply once it is completely dry.

1. Switch off the device and disconnect the power plug.
2. Prior to disinfection clean the device using a mild detergent as described above (see *Cleaning* on page 32).
3. Select a disinfecting method which meets the legal regulations and guidelines applicable to your area of application.
4. For example, use alcohol (ethanol, isopropanol) or disinfectants containing alcohol.
5. Wipe down the surfaces with a cloth you have moistened in disinfectant.
6. If the cuvette shaft needs to be removed for disinfection proceed for removal and assembly as described in the section on Cleaning (see *Cleaning the cuvette shaft cover* on page 32).
7. The removed cuvette shaft cover can be disinfected by spray disinfection.

7.3 Decontaminating before shipping

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

Warning! Risk to health from contaminated device

1. Follow the instructions in the decontamination certificate. It is available in PDF format on our homepage (www.eppendorf.com/decontamination).
2. Decontaminate all the parts you want to dispatch.
3. Enclose the fully-completed decontamination certificate for returned goods (incl. the serial number of the device) with the shipment.

7.4 Replacing fuses

Danger! Electric shock.

- Switch off the device and disconnect the power plug before opening the device to replace the fuses. These tasks may only be performed by appropriately trained staff.

1. Switch off the device and disconnect the power plug.
2. The fuse holder is above the mains connection **7** (see Fig. 1 on page 8). Press the small spring-loaded notch lever at the underside of the fuse holder and pull out the holder.
3. Replace the fuses (see order information in the operating manual).
4. Push the fuse holder back into its retainer until the notch lever engages.
5. Reconnect the power plug.

7 Maintenance

7.5 Check photometer

To check the photometric accuracy and the accuracy of the wavelength Eppendorf offers a filter kit (secondary UV-VIS filter). The kit contains three filters ("sample A1", "sample A2" and "sample A3") to check the photometric acccuracy and two filters ("sample 260 nm" and "sample 280 nm") to check the accuracy of the wavelength. The absorbances of the filters are measured against a blank filter ("blank A0"). In addition to information about the accuracy you are also provided with information about the precision: from the 10 measurements each per wavelength the CV value is calculated in addition to the mean value.

To perform a blank measurement, firstly blank filters are inserted like cuvettes into the cuvette shaft followed by the test filters. The absorbance values measured for the test filters are compared to the permitted value range. The limit values for the permitted range for the individual filters are printed in a table in the lid of the filter box (see in table: "X.XXX – X.XXX E").

Tab. 3: Inside lid of the filter box (sample)

BioPhotometer 6131 / 6132				**Function : PHOTOMETERTEST**		
Secondary / Sekundär - UV - VIS - Filter				Order No./Best.Nr.: 6131 928.007		
	Limits Grenzwerte	measured against **Blank A 0** at approx. 20°C gemessen gegen **Blank A 0** bei ca. 20°C				
6131	914.XXX	916.XXX	917.XXX	921.XXX	922.XXX	923.XXX
Filter Type	**Blank A 0**	**Sample 260 nm**	**Sample 280 nm**	**Sample A 1**	**Sample A 2**	**Sample A 3**
	Reference	Systematic error of wavelength Systematische Messabweichung der Wellenlänge traceable to NIST / rückführbar auf NIST		Systematic error of photometer Systematische Messabweichung des Photometers traceable to NIST / rückführbar auf NIST		
		Limiting values (A) / Grenzwerte (E)				
230 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
260 nm	0.000	X.XXX - X.XXX		X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
280 nm	0.000		X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
320 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
340 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
405 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
490 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
550 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
562 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
595 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
650 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX
		Random error of wavelength Zufällige Messabweichung der Wellenlänge		Random error of photometer Zufällige Messabweichung des Photometers		
		Limiting values CV (%) / Grenzwerte VK (%)				
230 - 650 nm		≤ 3.0 %		≤ 3.0 %	≤ 1.0 %	≤ 1.5 %
Please protect against dust, heat and liquid. The limits are valid for max. 2 years. Bitte vor Staub, Hitze und Flüssigkeiten schützen. Die Grenzwerte gelten für max. 2 Jahre.				Date Datum	Signature Unterschrift	**eppendorf**

7 Maintenance

7.5.1 Test procedure

- Carry out test at approx. 20 °C.
- Remove filter only briefly from the filter box and protect against contamination or damage to the filter surface.
- Protect filter against dust, heat, liquids and aggressive vapors.
- Always insert the filter with the sticker containing the filter designation facing the user (towards the recipient).

1. Open the function PHOTOMETER TEST / NEW MEASUREMENT.
2. Select the test filter and confirm the selection with "Enter".
3. "SAMPLE 260" or "SAMPLE 280" for measuring the accuracy of the wavelength for 260 or 280 nm.
4. "SAMPLE A1", "SAMPLE A2" or "SAMPLE A3" for measuring the photometric accuracy at 230, 260, 280, 340, 405, 490, 550, 595 and 650 nm.
5. Follow the instructions on the device display for measuring the blank ("A0") and the test filter. The device carries out 10 measurements each and then displays the mean value and the CV value for the measured absorbance at the respective wavelengths.
6. Press "Enter" in accordance with the instructions on the device display to have the values displayed and then printed - provided a printer is connected.
7. Compare the mean values and the CV values to the permitted ranges in the table supplied.

If the measured values do not match the permitted value range please contact Eppendorf Service. The filter should be recalibrated by Eppendorf after 2 years.

8 Troubleshooting

8.1 Result flags

Result flags	Possible cause	Remedy
0.015 A_{340}◀	Only for nucleic acid methods and "PROTEIN 280 nm": The marking '◀' of the A_{340} value indicates that the absorbance values for 230, 260 and 280 nm are corrected with the absorbance for 340 nm (parameter "CORRECTION A_{340}" is enabled).	None. The flag is only for information.
0.015 A_{550}◀ or 0.015 A_{650}◀	Only for method groups "Dye 550" and "Dye 650": The marking '◀' of the A_{550} value or the A_{650} value indicates that the absorbance values for 260 and 280 nm are corrected with the absorbance for 550 or 650 nm (parameter "CORRECTION$_{550\ (or\ 650)}$" is enabled).	None. The flag is only for information.
++++++	The measured absorbance is above A = 3.	▸ Dilute sample. ▸ Check cuvette volume (light path height is 8.5 mm). ▸ Clean cuvette shaft (see *Cleaning* on page 32) and insert cuvette the right way round: measuring surface towards the light path. The direction of the light path is indicated on the blue cuvette shaft cover by an arrow. ▸ Use cuvette from a material which is optically transparent for light of the measuring wavelength (for measurements in UV e.g.: Eppendorf UVette).
------	(Instead of a value for the ratio:) Ratio cannot be calculated because one of the absorbance values used for the calculation is A = 0 or A > 3.	Repeat measurement, if necessary with a diluted sample.

8 Troubleshooting

8.2 Error messages

Device displays with error messages can be exited using **Enter**.

If certain parameters are programmed incompletely or incorrectly the icon for the parameter key is shown in the display. In these cases press the **parameter**/**dilution** key instead. This brings you directly to the parameter list to correct the error.

Error text	Explanation / cause	Remedy
(Parameter) PLEASE PROGRAM e.g.: **FACTOR PLEASE PROGRAM**	Method parameter has not been programmed correctly or is incomplete.	▸ Check and correct parameter (see *Parameter* on page 26).
MEASURE BLANK FIRST	No blank has been stored for the method opened.	▸ Measure blank.
MEASURE STANDARD FIRST	No calibration has been stored for the method opened.	▸ Measure standards to save a valid calibration.
NO STD METHOD	The key **standard** has been pressed although no calibration evaluation has been programmed for the method.	▸ Program evaluation with standards in the measuring parameters or measure the method again without standards.
OUTSIDE OF CALIBRATION	Only for non-linear calibration evaluation: The measured sample absorbance is outside the absorbance range of the standard.	▸ Only measure samples which are within the calibration range. For too highly concentrated samples: dilute samples.
MEASURED VALUES NOT MONOTONIC	For multi-point calibration: The absorbance values of the standard do not result in a monotonic increasing or decreasing order.	▸ Check standards and measure again in the correct order (increasing concentration).
CALIBRATION CURVE IS NOT MONOTONIC	The calibration curve is not monotonic increasing or decreasing or does have a reversal point.	▸ Check standards, if necessary prepare again and re-measure.
PROGRAM STANDARDS INCREASING	For multi-point calibration the target concentration of the standards has not been programmed in increasing order.	▸ Program target concentration of the standards in increasing order.
CV IS GREATER THAN 10%	When calculating the calibration the "CV" is greater than 10%.	▸ Check standards, if necessary prepare again and re-measure.
CALIBRATION NOT OK!	The measured standard absorbances do not result in a valid calibration evaluation.	▸ Check standards and measure again.
MEASURED VALUES NOT PLAUSIBLE	Value cannot be displayed; potential causes when measuring with standards: • Standard for single point calibration has the absorbance "0" • Calculated factor for single point calibration is too great to be displayed. • Polynome for multi-point calibration cannot be displayed with the measured absorbance values.	▸ For measurements with standards: check measured absorbances; if necessary, prepare standards again and re-measure.
INVALID INPUT	When entering a sample number: The number "0" was entered	▸ Enter sample number between 1 and 999.
MEASURING MODULE FAULT 1 (or 2 or 3)	Technical error messages.	▸ Contact Eppendorf Service.

8 Troubleshooting

8.3 General errors

Fault	Possible cause	Remedy
Measuring results are imprecise.	• Shelf life of reagent solution exceeded.	▸ Ensure that the reagent is still within its shelf life and properly prepared.
	• Reagent not prepared correctly.	▸ Use clean demineralized water of adequate quality for preparation if required.
	• Pipetting incorrect.	▸ Ensure that the pipette is calibrated and that pipetting is being performed correctly.
	• Incubation procedure before measurement is incorrect.	▸ If the method procedure requires incubation before the measurement, ensure that the temperature and time for incubation are correctly observed.
	• Cuvette dirty.	▸ Clean and rinse the cuvette. When changing cuvette, ensure that the optical surface of the cuvette remains clean and has not come into contact with fingers. ▸ If the optical surface of the cuvette is soiled with fingerprints, clean it by wiping with a lint-free laboratory cloth soaked in alcohol.
	• Cuvette not completely full of measuring solution without bubbles.	▸ Ensure that the necessary minimum volume of the cuvette for a measurement is reached and there are no bubbles in the measuring solution.
	• Measuring solution turbid.	▸ Centrifuge turbid measuring solutions containing particles and use the clear supernatant.
	• Photometer drifting.	▸ Contact Eppendorf Service.
Measuring results incorrect.	• Method wrongly programmed.	▸ Ensure that the method parameters are entered correctly.
	• Standard solution not prepared correctly.	▸ Ensure that the correct standard is used and that the measuring solution for the standard is prepared correctly.
	• Reagent absorbance drifting.	▸ (With unstable reagent absorbance and end-point methods): when measuring a long series of samples, do not measure the reagent blank only at the beginning of the series, but also during the series of samples. If the reagent blank drifts significantly, the reagent is unsuitable for fault-free measurements and must be replaced with new reagent.
	Other possible causes can be found under "Measuring results imprecise".	Other possible remedies can be found under "Measuring results imprecise".

9 Transport, storage and disposal

9.1 Transport

- Only transport the device in the original packaging.

	Air temperature	Rel. humidity	Air pressure
General transportation	-25 to 60 °C	10 to 95%	30 to 106 kPa
Air freight	-40 to 55 °C	10 to 95%	30 to 106kPa

9.2 Storage

	Air temperature	Rel. humidity	Air pressure
in transport packaging	-25 to 55 °C	25 to 75%	70 to 106 kPa
without transport packaging	-5 to 45 °C	25 to 75%	70 to 106 kPa

9.3 Disposal

In the event of disposing of the product, please observe the applicable legal regulations.

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

The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC pertaining to waste electrical and electronic equipment (WEEE).

In accordance with this, any devices delivered after 13/08/2005 on a business-to-business basis, which includes this product, may no longer be disposed of in household waste. To document this they have been marked with the following identification:

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

10 Technical data

10.1 Power supply

Power supply	100 to 240 V ± 10 % / 50 to 60 Hz ± 5 %
Overvoltage category	IEC 61010-1 category II
Degree of contamination:	IEC 61010-1 category II
Power consumption	approx. 20 W during operation, approx. 10 W in standby mode
Permitted mains interruption	approx. 10 ms at 90 V approx. 20 ms at 220 V
Fuses	T 1A/250 V, 5 mm x 20 mm (2 off)

10.2 Ambient conditions

Operation	Ambient temperature: 15 to 35 °C Rel. humidity: 15 to 70% Atmospheric pressure: 86 to 106 kPa
Storage	Ambient temperature: -25 to 70 °C Rel. humidity: 15 to 70% Atmospheric pressure: 30 to 106 kPa

Not tropicalized.

Protect against direct sunlight.

10.3 Weight / dimensions

Weight	3 kg (packaged: 4.8 kg)
Dimensions	Width: 200 mm (packaged: 290 mm) Depth: 320 mm (packaged: 430 mm) Height: 100 mm (packaged: 200 mm)
Space required	Width: 400 mm (or 650 mm including printer) Depth: 500 mm

10.4 Interfaces

Interface for printer and PC	serial RS-232

The printer to be connected must meet the requirements of EN 60950 or UL 1950.

10 Technical data

10.5 Photometer

Measuring principle	Absorption single beam photometer with reference beam and several fixed wavelenghts
Light source	Xenon flash light
Monochromator	Holographic concave grating
Beam receiver	Silicon photo diodes
Wavelengths	230, 260, 280, 340, 405, 490, 550, 595, 650 nm
Wavelength selection	Dependent on method, controlled by program
Spectral bandwidth	5 nm at 230 to 340 nm 7 nm at 405 to 650 nm
Systematic wavelength error	± 1 nm at 230 to 280 nm ± 2 nm at 340 to 650 nm
Photometric measuring range	Quartz glass cuvette: • A = 0 to 3, except: • A = 0 to 2 at 340 nm • Only for dye methods: A = 0 to 2 at 550/650 nm UVette (Eppendorf): • A = 0 to 2.5 at 230 nm • A = 0 to 2.6 at 260 nm • A = 0 to 2.8 at 280 nm • Other values see quartz glass cuvette
Reading accuracy	ΔA = 0.001
Random photometric error	≤ 0.002 at A = 0 ≤ 0.005 at A = 1
Systematic photometric error	± 1% at A = 1
Stray light component	< 0.05 %

10.6 Other technical parameters

Cuvette material	For DNA, RNA, Oligo, Protein UV, Assay 340: Quartz glass or UV transparent plastic (UVette by Eppendorf) For OD600, Bradford, Lowry, BCA, Assay 405, Assay 490: Glass or plastic
Cuvette shaft	12.5 mm x 12,5 mm, not temperature-controlled
Overall cuvette height	Min. 36 mm
Height of the light beam in the cuvette	8.5mm
Light beam in the cuvette	Width: 1 mm Height: 1.5 mm
Keyboard	19 foil keys
Display	Illuminated graphic display, 33 mm x 60 mm
Operator guidance language	English, French, German
Result output	Via display and printer: Absorbance, concentration, ratio, FOI
Standards and regulations	According to VDE, CE, IEC 1010-1

10 Technical data

10.7 Application parameters

Method memory	32 preprogrammed, modifiable method programs
Measuring methods	End point against blank Dye methods: parallel measurement of biomolecule and dye label
Method-dependent evaluation	absorbance concentration via factor concentration via calibration with 1 to 10 standards: • single point calibration (1 standard) • linear regression (2 to 10 standards) • non-linear regression (polynome of 3rd degree; 4 or 5 to 10 standards (see *Evaluation procedure* on page 43)). • 1x, 2x or 3x measurement for nucleic acids: • ratio 260/280 • ratio 260/230 • molar concentration • total yield for dye methods: • FOI (frequency of incorporation; marking density)
Calibration memory	For all calibration methods
Measured value memory	For 100 results with abosrbance and ratio values, sample number, sample dilution, date and time

11 Evaluation procedure

This chapter describes the available evaluation procedures in the method programs (see *Parameter* on page 26) and the calculation of a dilution by the device software.

When comparing the measuring results to those of other photometers / spectrometers note that the values may depend on the bandwidths 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.

You should therefore check the accuracy of the method by measuring standards.

11.1 Evaluation with factor

$$C = E \times F$$

C = calculated concentration

A = measured absorbance

F = factor

The factor is programmed in the parameter list and can be modified. For the dye methods the factor for calculating the dye component is calculated from the absorbance coefficient of the device.

The factor always relates to the cuvette optical path length of 10 mm. If you change the parameter CUVETTE the modification is taken into account by the device 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.

11.2 Evaluation using standards

11.2.1 Single point calibration

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

F = calculated factor

C_S = nominal concentration of the standard

A_S = measured absorbance of the standard

The nominal concentration is programmed in the parameter list and can be modified.

If multiple measurement (2x, 3x) has been programmed for the standard, then the evaluation from the measured absorbances takes place via linear regression taking into account the zero value. After calculating the regression a CV value (coefficient of variation with a unit of %) is formed as measure for the scattering of the measured values. If the CV is greater than 10% it will be displayed. In this case the calibration will only be stored after confirmation.

The calculation of the sample concentration takes place using the calculated factor:

$$C = E \times F$$

11 Evaluation procedure

11.2.2 Multi-point calibration: calibration line

For the evaluation via a calibration line the selection "LINEAR" is programmed in the parameter "REGRESSION".

From 2 to 10 standards which can be measured once, twice or three times, a linear equation is calculated via linear regression (concentration as function of absorbance):

$$C = a_0 + a_1 E$$

a_0 = Interface of the calibration line with the concentration axis ("offset": concentration of a sample with absorbance 0)

a_1 = slope of the calibration line ("factor")

After calculating the regression the device forms a CV value (see above) as measure for the scattering of the measured values around the calibration line (exception: two point calibration with single measurement of both standards). If the CV is greater than 10% it will be displayed. In this case the calibration will only be stored after confirmation. For more than 2 standards the CV value is always displayed (even for a value below 10%).

In the function list the calibration data can be printed.

11.2.3 Multi-point calibration: Calibration curve

For the evaluation via a calibration curve the selection "NON-LINEAR" is programmed in the parameter "REGRESSION".

From 5 to 10 standards measured once, or 4 to 10 standards measured twice or three times, an equation for the third degree polynome (concentration as function of absorbance) is calculated via a non-linear regression.

$$C = a_0 + a_1 E + a_2 E^2 + a_3 E^3$$

a = coefficients (the coefficients are defined using the method of smallest squares)

After calculating the regression the device forms a CV value as measure for the scattering of the measured values around the calibration line (see above). If the CV exceeds 10% the calibration is only stored after confirmation.

The details above about the CV value and printing the calibration data apply accordingly.

11.3 Dilution

Dilutions entered into the method procedure are taken into account when calculating the results:

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

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

V_P = volume of the sample in the measuring solution

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

11 Evaluation procedure

11.4 Special evaluation procedures for the dye methods

11.4.1 Calculating the factor for the dye from the absorbance coefficient

In the dye methods a factor is calculated for the dye from the absorbance coefficient entered in the parameters and is displayed in the startup screen of the method procedure.

The factor is calculated as follows:

$$F_{d,Dye} = \frac{1}{\varepsilon_{Dye} \times d} \times 10^6$$

$F_{d, Dye}$ = factor for the dye with automatic allowance for the cuvette optical path length

ε_{Dye} = absorbance coefficient for the dye programmed in the method parameters; unit: $cm^{-1}M^{-1}$

d = cuvette optical path length, unit: cm

11.4.2 Calculation of the FOI

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

Unit DYE MOLECULE DYE / kb

$$FOI = \frac{E_{X50}}{\varepsilon_{Dye}} \times \frac{10^6 \times MM_{nt}}{E_{260} \times F}$$

Unit pmole/µg DNA (or RNA)

$$FOI = \frac{E_{X50}}{\varepsilon_{Dye}} \times \frac{10^9}{E_{260} \times F}$$

A_{X50} = measured absorbance of the dye at 550 or 650 nm

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

MM_{nt} = average molecular mass of the nucleotides; unit: g/mol; for dsDNA/ssDNA/Oligo DNA: 325; for RNA / Oligo RNA: 337

A_{260} = measured absorbance of the nucleic acid

F = factor for calculating the nucleic acid programmed in the method parameters. The factor relates to the cuvette optical path length of 10 mm and does not need modifying if the parameter CUVETTE is modified.

11 Evaluation procedure

11.5 Special evaluation procedures for nucleic acids and protein UV

This section relates to the evaluation of nucleic acids or proteins in the methods dsDNA, ssDNA, Oligo DNA, RNA, Oligo RNA, Protein UV and the corresponding biomolecule components in the dye methods.

11.5.1 Correction A_{340}

Application: Partial correction of distortions of absorbance due to turbidity in the measuring solution.

The application of the evaluation procedure can be activated in the parameter list of the method.

$$E_{X,korr} = E_X - E_{340}$$

$A_{X,\ corr}$ = calculated corrected absorbance at a wavelength of 230, 260 and 280 nm

A_X = measured absorbance at a wavelength of 230, 260 and 280 nm

A_{340} = measured absorbance at a wavelength of 340 nm

11.5.2 Correction $A_{550/650}$

Application: Correction of the effect of the dye absorbance on the nucleic acid or protein absorbance at 260 and 280 nm.

The application of the evaluation procedure can be activated in the parameter list of the method.

$$E_{X,korr} = E_X - KF_X \times E_{X50}$$

$A_{X,\ corr}$ = calculated corrected absorbance at a wavelength of 260 and 280 nm

A_X = measured absorbance at a wavelength of 260 and 280 nm

KF_X = correction factor for the wavelength 260 or 280 nm (the two correction factors for 260 and for 280 nm are programmed in the parameter list of the method)

A_{X50} = measured absorbance at a wavelength of 550 (or 650) nm

---

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

11.5.3 Conversion into molar concentrations and nucleic acid amounts

The conversion can only be applied to nucleic acids and dye methods with nucleic acids as biomolecule component.

Calculation of amount

Application: Calculation of the amount (mass) of nucleic acid or nucleic acid dye conjugate in the whole sample volume.

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

M = calculated total amount (mass) of the nucleic acid or the conjugate in the sample tube

C = calculated concentration of the nucleic acid or the conjugate

$V_{P,\ total}$ = volume of the sample in the sample tube; entered by the user in the third screen of the result display (see *Nucleic acids* on page 19).

11 Evaluation procedure

Calculation of the molar concentration

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

$$C_{Mol} = \frac{C}{MM}$$

C_{Mol} = calculated molar concentration of the nucleic acid; the unit for the molar concentration is programmed in the parameter list of the method; dependent on the programmed unit the formula above is adjusted automatically.

C = mass concentration of the nucleic acid in ng/µl or µg/ml, from the measured absorbance the device calculates the concentration result and displays it on the results screen

MM = relative molar mass in kDa

If in the third results screen the number of bases or base pairs per nucleic acid molecule has been entered instead of the relative molar mass, then the molar mass is calculated from the number of bases (or 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 in kDa

bp = entered number of base pairs per molecule

b = entered number of bases per molecule

12 Ordering information

Order No. (International)	Order No. (North America)	Description
6132 000.008 6132 000.016	- 952000006	**BioPhotometer plus** 230 V / 50-60 Hz, power plug Europe, more types of mains connection available 120 V / 50-60 Hz, power plug North America
6131 928.007	952010221	**Secondary UV-VIS-Filter** Test filter set for checking photometric accuracy und wavelength accuracy (according to NIST)
6131 011.006 6131 010.000	- 952010140	**Thermal Printer DPU 414** incl. power supply and printer cable 230 V 115 V
0013 021.566	952010409	**Thermo paper** 5 rolls
0030 106.300	952010051	**UVette®** individually packaged single cuvettes, directly usable with BioPhotometer, certified RNase-, DNA- and protein-free 80 cuvettes
0030 106.318	952010069	**UVette® routine pack** Eppendorf Quality purity level, reclosable box 200 cuvettes
4308 078.006	940001102	**Cuvette stand** for 16 cuvettes

EG-Konformitätserklärung
EC Conformity Declaration

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

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

Produktbezeichnung, Product name:

BioPhotometer plus 6132

Produkttyp, Product type:

Photometer

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

2006/95/EG, EN 61010-1

2004/108/EG, EN 55011/B, EN 61000-6-1, EN 61000-3-2, EN 61000-3-3, EN 61000-4-14

Vorstand, Board of Management:

29.10.2007

Hamburg, Date:

Projektmanagement, Project Management:

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