BioPhotometer

Bedienungsanleitung
Operating Manual
Mode d'emploi
Istruzioni d'impiego
Manual de Instrucciones

eppendorf

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1 Device display
2 9 method keys
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The main power key, main power connection and printer connection are located on the rear of the device (see Section 4, "Installation").

The BioPhotometer from Eppendorf is used for rapid, simple and convenient measurement of the most common methods in research labs in the fields of molecular biology and biochemistry.

Cuvettes

Standard rectangular cuvettes made of glass or plastic that transmit light at every measuring wavelength may be inserted into the cuvette shaft. Using the UVette® from Eppendorf, it is now possible to measure nucleic acids in a plastic cuvette.

The height of the measuring window (8.5 mm), as well as the total height (min. 36 mm) must be taken into consideration when the cuvettes are selected (see chap. 10 "Short instruction"). To ensure correct, precise results, please ensure that the cuvettes are clean and that the measuring solution is particle-free. A seal is included with the device to protect the cuvette shaft from dust when not in use.

1 Overview

Methods

There are twelve preprogrammed factory-set methods which can be called up at the push of a button:

Nucleic acids	
dsDNA	Double-stranded DNA
ssDNA	Single-stranded DNA
RNA	RNA
Oligo	Oligonucleotides
Proteins	
Protein	Direct photometric measurement
Bradford	Bradford method
Bradford micro	Bradford method, low concentration range
Lowry	Lowry method
Lowry micro	Lowry method, low concentration range
BCA	BCA method
BCA micro	BCA method, low concentration range
Bacteria density	
OD 600	Turbidity measurement

Method

Each method has an accompanying, factory-set program that contains different parameters, such as units of concentration and type of calculation. The method programs can be changed at any time using the Parameter key. Before using a method for the first time, call up the corresponding method program and – if necessary – adapt it to suit your requirements. For methods which are to be calculated using calibration by standard measurements, the number and nominal concentrations of the standards must be adapted.

Measurement

For measurement purposes, the desired method should be called up using the appropriate measuring key. The Bradford, Lowry and BCA methods have the same special feature: For each of these methods, two different calculation ranges may be programmed. It is possible to toggle between the two method programs (e.g. "BCA" and "BCA micro") by pressing the method key repeatedly.

Pressing one of the three oval measuring keys starts the measurement. The device is ready to measure immediately after being switched on. An indication as to which of the three measuring keys should be used for a measurement can be found in the lower part of the device display (Details on the measuring process can be found in Section 5, "Operation").

Calculation

It is possible to calculate the result automatically using method-specific programmed calculation modes (factor, calibration, Warburg formula or direct absorbance output). In addition to the calculated results, the absorbances and (for nucleic acids) the common absorbance ratios appear in the display.

Sample dilutions can also be included in the calculation process (Dilution key). The calculated mass concentrations for nucleic acids can be converted into molar concentrations by pressing the Conversion key. This key can also be used to calculate the total sample quantity ("yield") in the sample vessel.

Results printout

The results appear in the device display and can be printed out (if the printer is connected). A data transfer program is available from Eppendorf for evaluating your results on a computer using a calculation program (see Sec. 11, "Ordering information").

Sample results and calibration results are stored; this data can be called up by pressing the Function key.

2 Technical data

Photometer

Optical system:	Absorption single-beam photometer with reference beam and several fixed wavelengths
Irradiation source:	Xenon flash lamp
Spectral dispersion:	Holographic concave grating
Measuring wavelengths:	Xe 230, 260, 280, 320, 562, 595 nm
Wavelength selection:	Method-dependent, program-controlled
Spectral bandwidth:	5 nm at 230 to 320 nm 7 nm at 562 to 595 nm
Wavelength systematic error:	± 1 nm at 230 to 280 nm ± 2 nm at 320 to 595 nm
Wavelength random error:	≤ 0.1 nm
Photometric measuring range:	Quartz glass cuvette: 0.000 to 3.000 A UVette® (Eppendorf): 2.5 A at 230 nm 2.6 A at 260 nm 2.8 A at 280 nm 2.9 A at 320 nm
Photometric random error:	≤ 0.002 A at 0 A ≤ 0.005 A at 1 A
Photometric systematic error:	± 1 % at 1 A
Accuracy of reading:	0.001 A
Stray-light proportion:	< 0.05 %
Radiation detector:	Silicium photo diodes

Measuring procedures

Measuring procedure:	End-point against blank
Method-dependent calculation:	Absorbance Concentration via factor Concentration via Warburg formula Concentration via calibration with 1 to 10 standards One-point calibration (1 standard) Linear regression (2 to 10 standards) Non-linear regression (3rd degree polynomer; 4 or 5 to 10 standards; see Section 12, "Calculation") 1 x, 2 x or 3 x determination For nucleic acids: Ratio 260/280 Ratio 260/230 Molar concentration Total yield

Memory

Method memory:	12 preprogrammed, modifiable method programs
Calibration memory:	For all calibration procedures
Results memory:	For 100 results with absorbance and ratio values, sample number, sample dilution, date and time (calendar up to 2090)

2 Technical data

Operation

Cuvette material:	dsDNA, ssDNA, RNA, Oligo, Protein: Quartz glass or plastic (UVette® from Eppendorf) OD 600, Bradford, Lowry, BCA: Glass or plastic
Cuvette shaft:	12.5 mm x 12.5 mm, not temperature-controlled
Overall height of cuvettes:	Min. 36 mm
Height of light beams in the cuvette:	8.5 mm
Light bundle in the cuvette:	Width: 1 mm Height: 1.5 mm
Keypad:	19 foil keys
Display:	Illuminated graphic display, 33 mm x 60 mm
User guidance:	English, French, German
Results output:	Via display and printer Absorbance, concentration, ratio

General data

Supply voltage:	100 to 240 V ± 10 %; 50 to 60 Hz ± 5 %
Overvoltage category:	II (IEC 61010-1)
Pollution degree:	2 (IEC 664)
Power requirement / power output:	Approx. 20 W in operation, approx. 10 W in Standby mode
Current consumption:	< 0.3 A
Permitted mains interruption:	Approx. 10 ms at 90 V Approx. 200 ms at 220 V
Fuses:	T 1 A / 250 V, 5 mm x 20 mm (2 pcs.)
Ambient conditions:	15 to 35 °C with defined precision and accuracy –25 to 70 °C when not in operation or when stored 15 to 70 % relative humidity Cannot be used in tropical climate Keep out of direct sunlight
Printer connection:	RS-232 C, serial, data format: 1 start bit, 8 data bits, no parity, 1 stop bit, 9600 Baud The printer that is connected must comply with the requirements of EN 60950 or UL 1950.
Standards and regulations:	Complies with VDE, CE, IEC 1010-1
Dimensions:	Width: 20 cm (packaged: 29 cm) Depth: 32 cm (packaged: 43 cm) Height: 10 cm (packaged: 20 cm)
Weight:	3 kg (packaged: 4,8 kg)

Technical specifications subject to change.

3 Safety precautions and prevention of damage

Before using the Biophotometer please familiarize yourself completely with the operating instructions. The following points must be followed exactly to enable safe work with the device:

Technical safety

- Do not open the device.
- Do not allow any liquid to enter into the device.
- Disconnect the device from the mains supply before carrying out maintenance work or changing the fuses.
  The inside of the device is a high-voltage area. Danger!
- Do not operate the device in a hazardous location or potentially explosive environment.
- Do not use the device if it is damaged, especially if the main power cable is in any way damaged or defective.
- Repairs may only be carried out by the service technicians from Eppendorf AG and by authorized contractual partners.
- The device must be connected to a power outlet that has a protective ground connection.
- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Handling biological and chemical material

- Reagents and dilution buffers can cause cauterization and other damage to health.
- Samples (nucleic acids, proteins, bacteria cultures) can be infectious and cause serious damage to health.
- During sample preparation, measuring procedures and maintenance and cleaning work, observe all local laboratory safety precautions (e.g. wear protective clothing and gloves, use of disinfectant) regarding the handling of sample material.
- Dispose of measuring solutions and cleaning and disinfectant materials in accordance with the relevant local laboratory regulations.

Transfer

- If the device is passed on to someone else, please include the instruction manual.

Disposal

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

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

- The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC on waste electrical and electronic equipment (WEEE).
- According to these regulations, any devices supplied after 13.08.05 in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. They are marked with the following symbol to indicate this.
-  As disposal regulations within the EU may vary from country to country, please contact your supplier if necessary.

4 Installation

Delivery package

- BioPhotometer
- Mains cable for BioPhotometer
- Operating manual, incl. short instructions
- Seal for cuvette shaft

4.1 BioPhotometer

Connect up device

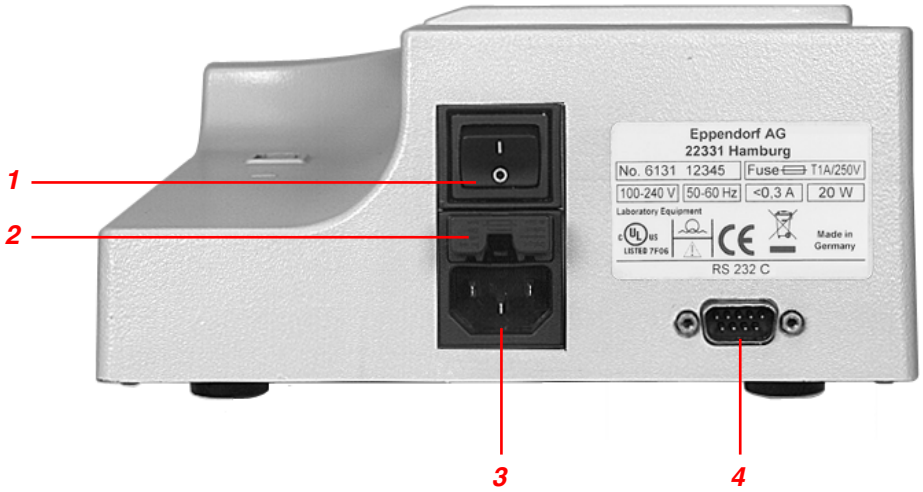
Space required: Width: 40 cm
Depth: 50 cm

Power connection: Safety socket

- Insert the mains plug of the device into the safety socket.
  It is not necessary to set voltage of the device within the voltage range specified in "Technical data" because the voltage is set automatically within this range.

Ambient conditions: see "Technical data".

- Remove the protective foil from the device display.



1 Mains switch
2 Fuse holder
3 Mains connection
4 Printer connection, serial (RS-232 C)

4.2 Printer

Printer DPU 414

The Eppendorf Thermal Printer DPU 414 can be connected to the serial interface RS-232 C of the BioPhotometer (see Section 11, "Ordering information").

– Insert the printer cable into the printer connection socket of the BioPhotometer (see photo) and tighten the safety screws on the plug to secure.

– Connect the printer cable to the printer and tighten the safety screws on the plug to secure.

– Connect up to the power supply using a 115 V or 230 V mains cable.

Setting the printer function

BioPhotometer

– Select the function "Printer DPU 414" in the function list, and confirm.

Printer DPU 414

– Check the printer settings. If necessary, set the printer for use with the BioPhotometer, as described in the printer supplement.

Printer settings for working with the BioPhotometer:

Dip SW-1

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

Settings made by the user are not relevant for the group "Dip SW-2" because the BioPhotometer assumes these settings automatically in accordance with the language version selected.

Dip SW-3

1 (ON) : Data Length = 8 bits
2 (ON) : Parity Settings = No
3 (ON) : Parity Conditions = Odd
4 (OFF) : Busy Control = XON/XOFF
5 (OFF) : Baud
6 (ON) : Rate
7 (ON) : Select
8 (ON) : =9600 bps

Other printers

In addition to the DPU 414, it is also possible to connect other serial printers to the serial interface of the BioPhotometer. With the aid of an adapter cable, parallel printers can also be connected.

BioPhotometer

– Select the function "Printer serial" in the functions list, and confirm.

Printer

Requirements for the serial printer:

Busy Control	:	XON/XOFF
Baud Rate(ON)	:	9600 bps
Data Bit Length	:	8 bits
Parity Permission	:	Without
Parity Conditions	:	Odd

Parallel printers can be connected using an adapter cable which fulfills the above requirements.

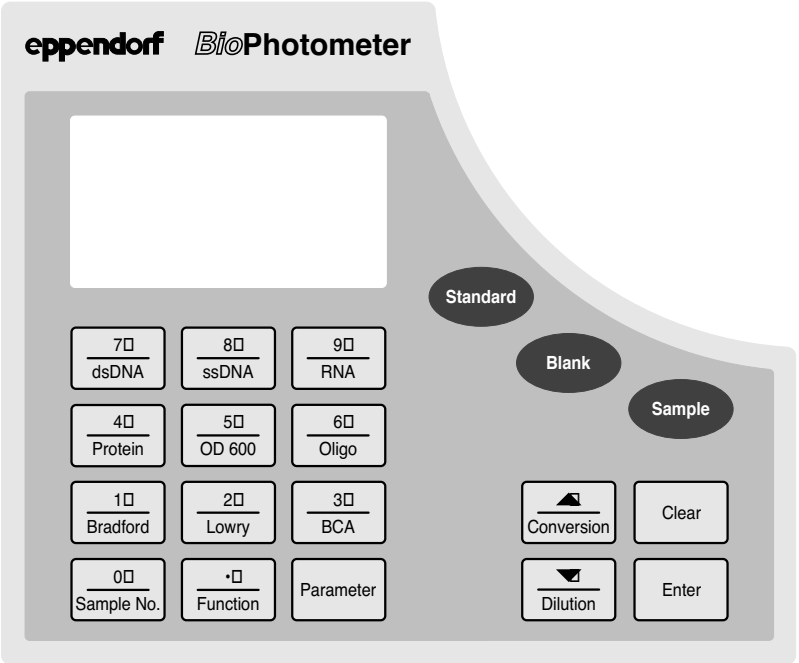
4.3 Cuvettes

Commercially available rectangular cuvettes may be used in the cuvette shaft. When the height of the measuring window is 8.5 mm above the cuvette base and the overall height of the cuvette is at least 36 mm (see the graphics in "Short instructions"). The light bundle in the cuvette is 1.0 mm wide and 1.5 mm high.

For measurements, cuvettes made of glass or plastic may be used on condition that they are transparent at the respective measuring wavelength. The UVette® from Eppendorf is a plastic cuvette which is transparent at wavelengths as low as 220 nm, which means that it is also suitable for nucleic acid measurement.

5 Operation

5.1 Keypad



7□ dsDNA

- To call up the "Double-stranded DNA" method.
- To enter figure 7.

8□ ssDNA

- To call up the "Single-stranded DNA" method.
- To enter figure 8.

9□ RNA

- To call up the "RNA" method.
- To enter figure 9.

6□ Oligo

- To call up the "Oligonucleotide" method.
- To enter figure 6.

4□ Protein

- To call up the "Protein (direct photometric measurement)" method.
- To enter figure 4.

1□ Bradford

- To call up the "Bradford" and "Bradford micro" methods.
- To switch between the "Bradford" and "Bradford micro" methods.
- To enter figure 1.

2□ Lowry

- To call up the "Lowry" and "Lowry micro" methods.
- To switch between the "Lowry" and "Lowry micro" methods.
- To enter figure 2.

5 Operation

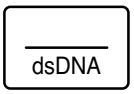
3□ / BCA	– To call up the "BCA" and "BCA micro" methods. – To switch between the "BCA" and "BCA micro" methods. – To enter figure 3.
5□ / OD 600	– To call up the "OD 600 (measuring the bacteria density)" method. – To enter figure 5.
Parameter	– To call up the programming level. – To exit the programming level.
•□ / Function	– To call up the function level. – To exit the function level. – To enter a point.
0□ / Sample No.	– To change the sample number. – To enter figure 0.
▼ / Dilution	– To enter the dilution. – To move the cursor to the next line. (e.g. in the parameter list or function list).
◢ / Conversion	– To calculate the molar concentration and the total amount of sample ("yield"). – To move the cursor to the previous line. (e.g. in the parameter list or function list).
Clear	– To delete entries.
Enter	– To confirm entries.
Standard	– To measure a standard.
Blank	– To measure a blank.
Sample	– To measure a sample.

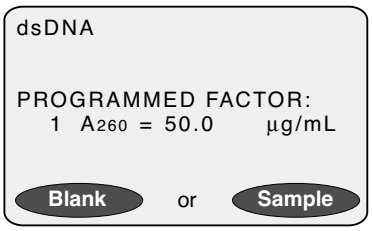
5.2 Measuring nucleic acids

This description is valid for the following methods:

- dsDNA
- ssDNA
- RNA
- Oligo

Call up method





Calculation

The factory-set factors are those which are normally used with nucleic-acid methods for the conversion of UV absorbance into concentration (in this example: 50). The factors can be changed using the Parameter key (see "Programming"). The number of decimal places of the result is determined by the number of decimal places of the programmed factor.

If a unit of concentration other than µg/mL is selected (e.g. µg/µL), the BioPhotometer converts the factor internally in order to produce the correct result.

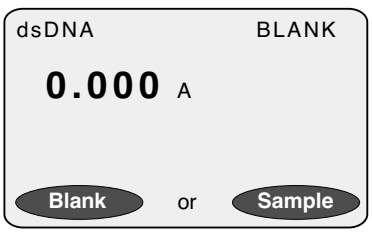
Measuring solutions with absorbances lower than app. 0.02 to 0.03 A_{260} (corresponds to a DNA concentration of app. 1.0 to 1.5 µg/mL) should not be used. This is because with such low absorbance, disturbances such as small particles, micro-bubbles or turbidity have a great deal of influence upon the measuring result and often lead to unreliable results.

Measuring procedure

Blank measurements remain stored until the date changes. If a blank has already been measured on the same day, the BioPhotometer offers the following in the last line of the display after method call-up:

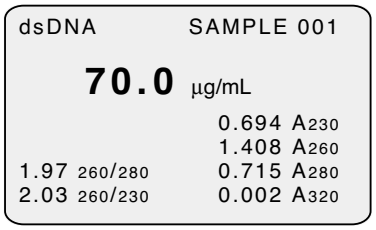
- To measure a new blank ***or***
- To measure a sample directly and to use the stored blank. If no blank has been measured on the same day, the instrument will only allow blank measurement.

Measure blank



Measure sample

Sample



Results display

As an indication of the purity of the nucleic acid sample which has been measured, the absorbance at 230, 280 and 320 nm as well as the ratios A_{260}/A_{280} and A_{260}/A_{230} are displayed in addition to the concentration result and the absorbance at a wavelength of 260 nm. With pure samples, the absorbance at 320 nm should be approximately zero.

Turbid measuring solutions show increased absorbances for all wavelengths. These can adulterate the results. In such cases the influence on the result can be partially corrected by switching on the "Corr. with A_{320}" parameter. (Chapter 6.3 "Explanation of parameters")

Measure next sample

To measure the next sample, press the Sample key again.

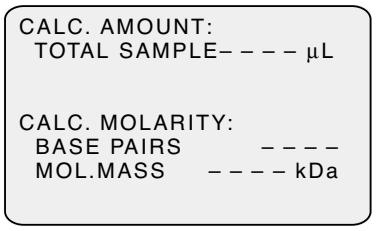
Sample dilution

The sample dilution in the measuring cuvette can be entered using the Dilution key before the measurement starts and is included automatically in the result calculation (see "Measuring diluted samples").

Conversion key

The most-recently measured concentration result can be converted into molar concentrations and/or into nucleic acid quantities (unit of mass or unit of mol):

Conversion



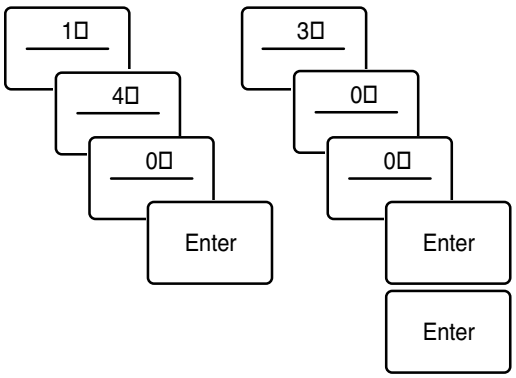
Entering "TOTAL SAMPLE"

The value entered is converted using the concentration measured. The result shown is the quantity of nucleic acid present in the sample.

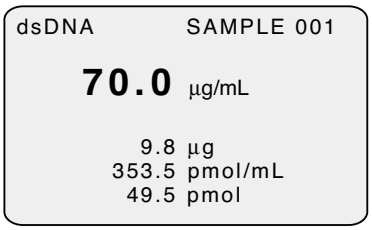
Entering "BASE PAIRS" or "MOL.MASS"

It is sufficient to make an entry in only one of the two lines. The molar concentration is calculated using the value entered and the concentration measured.

Input fields can be skipped using the Enter key.



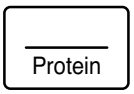
Display after entry of "140 µL sample volume" and "300 base pairs":

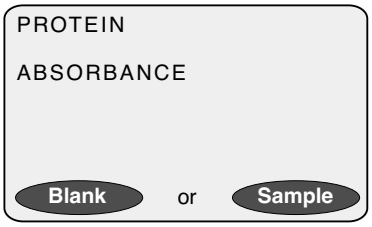


The molar unit of concentration (here: "pmol/mL") is preprogrammed, but can be selected and changed using the Parameter key.

5.3 Direct photometric measurement of protein

Call up method





Calculation

For the "protein" method, the "absorbance" calculation mode is stored, i.e. only the absorbances which are measured directly appear in the display. Calculations via the following calculation procedures can be programmed using the Parameter key (see Section 6 "Programming"):

- Factor
- Standard (One-point calibration)
- Warburg formula

The number of decimal places of the preprogrammed factor or the preprogrammed nominal concentration of the standard determines the number of decimal places in the result.

When programming the factor, please ensure that the factor is adapted in line with the unit of concentration selected.

Measuring solutions with absorbances lower than app. 0.02 to 0.03 A_{280} corresponds to a DNA concentration of app. 1.0 to 1.5 µg/mL should not be used. This is because with such low absorbances, disturbances such as small particles, microbubbles or turbidity have a great deal of influence upon the measuring result and often lead to unreliable results.

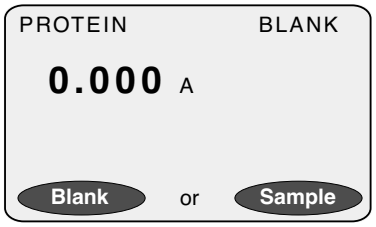
Measuring procedure

The following example shows the measuring procedure for the "Absorbance" calculation mode. For details of the measuring procedure via standard (one-point calibration), please refer to "Measuring proteins with reagent".

Blank measurements remain stored until the date changes (For further details, please refer to "Measuring nucleic acids").

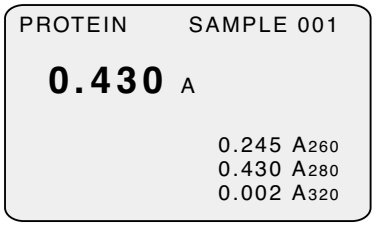
Measure blank

Blank



Measure sample

Sample



Results display

In addition to the concentration result and the absorbance at the measuring wavelength of 280 nm, A_{260} and A_{320} appear in the display as an indication of the purity of the sample. The absorbance at 320 nm should be approximately zero.

Turbid measuring solutions show increased absorbances for all wavelengths. These can adulterate the results. In such cases the influence on the result can be partially corrected by switching on the "Corr. with A_{320}" parameter. (Chapter 6.3 "Explanation of parameters")

Measure next sample

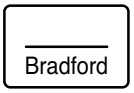
To measure the next sample, press the Sample key again.

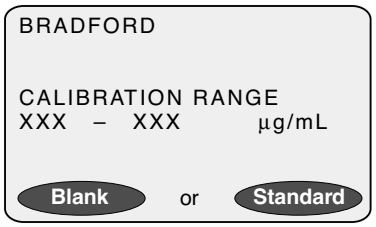
Sample dilution

Sample dilution in the measuring cuvette can be entered using the Dilution key before the measurement begins and is then included automatically in the following calculation of sample concentrations (see "Measuring diluted samples").

5.4 Measuring proteins with reagent (Bradford, BCA, Lowry)

Call up method





If a valid calibration (which is then stored by the device) has already been performed, the date and time of the stored calibration appear. In this case, the method can be recalibrated after blank measurement or the sample measurements may begin directly and can then be calculated using the previously stored calibration.

Micro methods

The Bradford, Lowry and BCA methods have a special feature: Two different concentration ranges may be programmed for each of these methods. It is possible to toggle between the two methods (e.g. "BCA" and "BCA micro") by pressing the method key repeatedly.

Calculation

For the Bradford, Lowry and BCA methods, the device contains a factory-set calibration procedure via multiple-point calibration and calculation of a calibration curve via non-linear regression. Other calculation methods may be programmed using the Parameter key (see Section 6 "Programming"):

- Factor (calculation of concentration values via factor).
- Absorbance (the measured values appear as absorbance values with no further calculation).

The following parameters may be changed for the factory-set calculation procedure via standard (see "Programming").

- Number of standards (1 to 10).
- Number of multiple measurements per standard (1 to 3).
- Calculation procedure for multiple-point calibration (linear or non-linear calibration).
- Nominal concentrations of the standards.

The number of decimal places of the preprogrammed factor or the preprogrammed nominal concentration of the standard determines the number of decimal places in the result.

In the case of calculation via factor, please ensure that the factor is adapted in line with the unit of concentration selected.

Measuring procedure

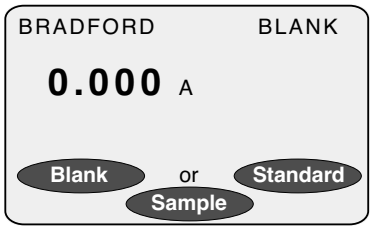
Blank measurements remain stored until the date changes (For further details, see "Measuring nucleic acids").

Standard measurements remain stored until they are overwritten with new standard measurements. For the calculation of sample measurements, the most-recently stored calibration is used.

In the following example, multiple-point calibration with 5 standards in double determination and calibration calculation via non-linear regression was programmed as a calculation procedure for the Bradford method:

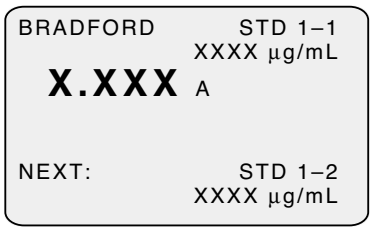
Measure blank

Blank



Measure standards

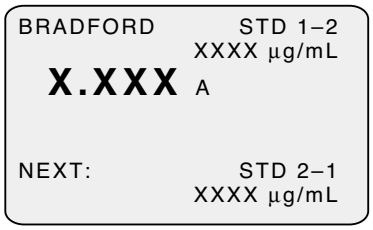
Standard



Standard 1 / first measurement

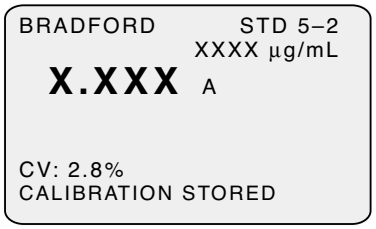
The first two lines of the display contain the standard that has just been measured. The last two lines of the display contain the next standard which is to be measured, with the nominal concentration.

Standard



Standard 1 / second measurement

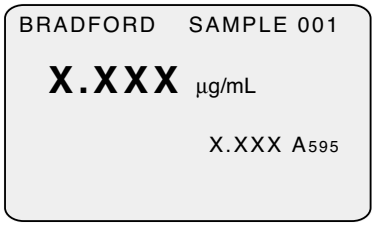
Device display following all standard measurements:



The CV (coefficient of variation) is a measure of the scattering of standard values around the regression curve. If the CV is smaller than 10 %, the calibration is stored automatically. If the CV is greater than 10 %, the question "STORE? ENT/CLR" appears, and you may then accept or delete the calculated calibration. Sample measurements are calculated using the most-recent valid calibration.

Measure sample

Sample



Results display
In addition to the concentration result, the absorbance at the respective wavelength (for Bradford: 595 nm) appears in the display.

Measure next sample

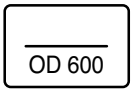
To measure the next sample, press the Sample key again.

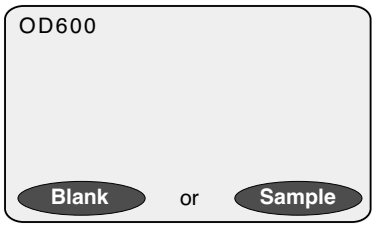
Sample dilution

Sample dilution in the measuring cuvette can be entered using the Dilution key before the measurement begins and is then included automatically in the result calculation (see "Measuring diluted samples").

5.5 Measuring OD 600

Select method

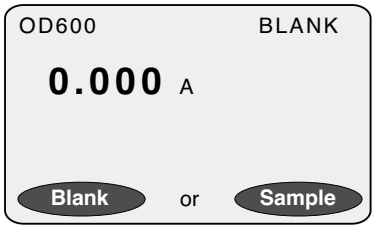




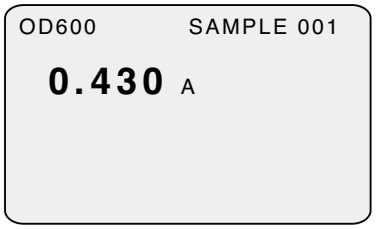
Measuring procedure

Blank measurements remain stored until the date changes (for further details, see "Measuring nucleic acids").

Measure blank



Measure sample



Measure next sample

To measure the next sample, press the Sample key again.

Sample dilution

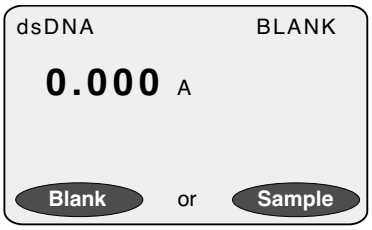
Sample dilution in the measuring cuvette can be entered using the Dilution key before the measurement begins and is then included automatically in the result calculation (see "Measuring diluted samples").

The OD 600 measurement is a stray-light measurement; the result is therefore heavily dependent on the geometry of the light path, which may vary between photometers from different manufacturers.

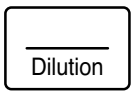
5.6 Measuring diluted samples

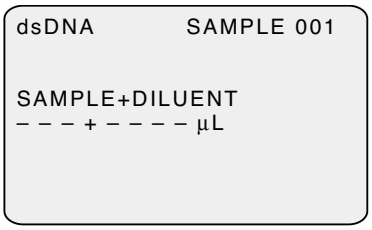
Sample dilutions may be entered using the Dilution key before the measurement begins. When the result is calculated and displayed, the dilution factor is included automatically.

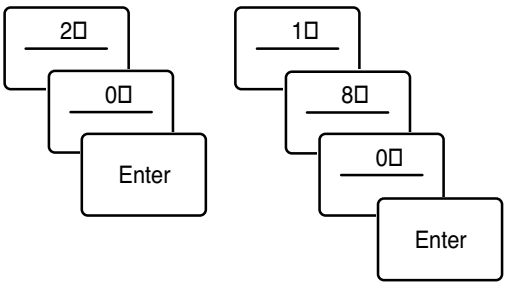
In the following example, a blank has already been measured:

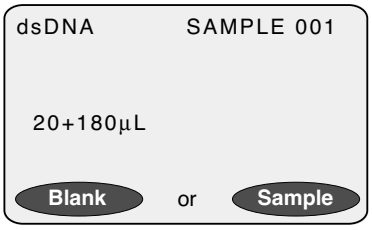


Enter dilution

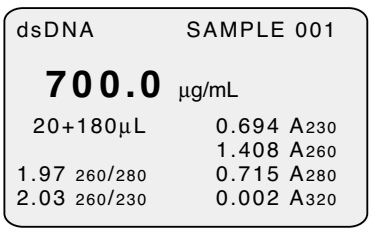








Measure diluted sample



The sample dilution is included in the result. The dilution factor entered remains stored for the calculation of further sample results until it is overwritten.

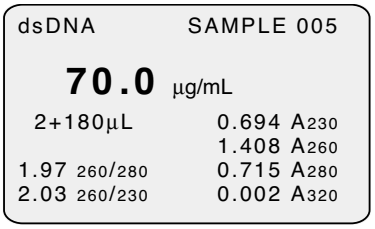
Deleting dilution entry

To delete the dilution factor, press the Dilution key again. The values for "Sample" and "Diluent" are then deleted using the Clear key or are overwritten with "zero".

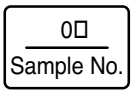
5.7 Changing the sample number

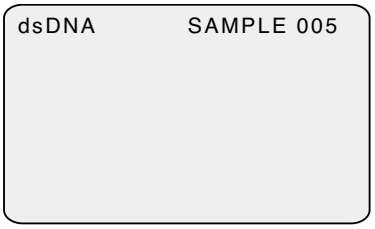
During sample measurements, the serial number of the sample appears in the top right of the display. The sample number is counted separately for each method and is reset to "1" when the date changes.

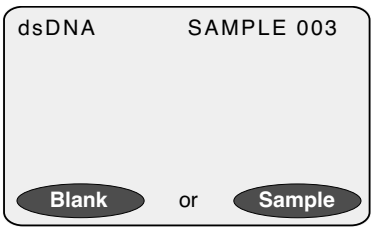
The sample number can be changed as desired (e.g. for repeat measurements):



Change sample number







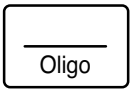
For the next sample to be measured, the sample number was set to "3". Additional samples are counted serially from the newly-entered number onwards.

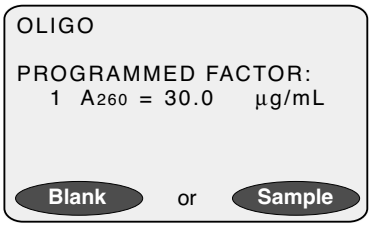
6 Programming

6.1 Programming procedure

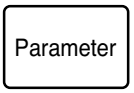
For each method, parameters such as the type of calculation or the unit of concentration are stored. The factory-set method programs can be changed using the Parameter key.

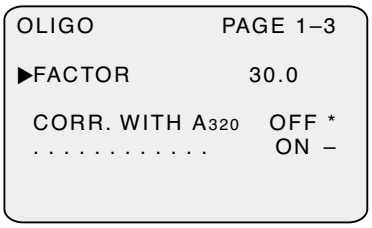
Call up method





Call up parameter list



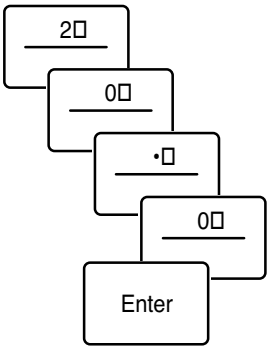


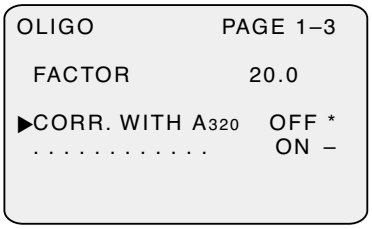
There are different parameter lists for the various different methods, all of which can be modified (see Section 6.2 for overview). The parameters for the "Oligo" methods extend across three pages of the device display.

Example: Changing the factor

Any numbers that are entered are stored by pressing Enter:

Enter factor and store



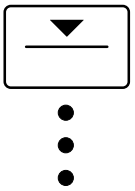


After the factor has been stored, the cursor moves to the next parameter-selection block ("Correction with A320").

Example: Changing the unit

Selection parameters are selected using the cursor keys and confirmed by pressing [Enter]. The stored setting is marked with an asterisk (*):

Select parameter



```
OLIGO              PAGE 2–3

 UNIT                µg/mL *
 . . . . . .          ng/µL –
▶. . . . . .          µg/µL –

 M. UNIT           pmol/µL *
 . . . . . .         µmol/L –
```

Store parameter

[Enter]

```
OLIGO              PAGE 2–3

 UNIT                µg/mL –
 . . . . . .          ng/µL –
 . . . . . .          µg/µL *

▶M. UNIT           pmol/µL *
 . . . . . .         µmol/L –
```

After the unit of concentration"µg/µL" has been stored, the cursor moves to the next selection block ("molar unit").

Exit parameter level

To exit the parameter level, select the line "PARAMETER END" and press [Enter]. Alternatively, press the [Parameter] key from any parameter line.

[Parameter]

```
OLIGO

PROGRAMMED FACTOR:
  1  A260 = 20.0    µg/mL

 Blank     or     Sample
```

6.2 Overview of parameters

	dsDNA ***ssDNA*** ***RNA***	***Oligo***	***Protein***	***Bradford*** ***Brad.micro*** ***Lowry*** ***Low.micro*** ***BCA*** ***BCA micro***	***OD 600***
Calculation	*(Point 1)*	*(Point 1)*	Absorbance Standard Factor Warburg formula	Absorbance Standard Factor	*(Point 1)*
Correction with A320	Off On	Off On	Off On		
Unit	µg/mL ng/µL µg/µL	µg/mL ng/µL µg/µL	mg/mL µg/mL	mg/mL µg/mL µg	*(Point 2)*
Molar unit	pmol/µL µmol/L pmol/mL	pmol/µL µmol/L			
Cuvette	10 mm 5 mm 2 mm 1 mm	10 mm 5 mm 2 mm 1 mm	10 mm 5 mm 2 mm 1 mm	10 mm 5 mm 2 mm 1 mm	10 mm 5 mm 2 mm 1 mm

(For "Factor" calculation only:)

Factor	Entry of numbers	Entry of numbers	Entry of numbers	Entry of numbers	Entry of numbers

(For "Standard" calculation only:)

No. of standards			*(Point 3)*	Entry of numbers	
Std. measurement			1x 2x 3x	1x 2x 3x	
Regression *(Point 4)*				Linear Non-linear	
Standard			Entry of numbers	Entry of numbers	

Point 1: *No selection possible; "Factor" calculation is preprogrammed.*

Point 2: *No selection possible; the unit "Absorbance" is preprogrammed.*

Point 3: *No selection possible; the number of standards "1" is preprogrammed.*

Point 4: *Selection possible only if at least "4" (or, for the single determination of the standard, at least "5") has been entered for the "Std. number" parameter.*

6.3 Explanation of parameters

Parameters are defined as selection parameters or as parameters for entering numbers. In the case of selection parameters, the programmable alternatives are method-dependent (see overview in previous section).

Parameter	*Entries*	*Explanation*
Calculation	Selection	Selection of calculation procedures: Absorbance, Factor, Standard and Warburg formula. In the case of calculation using the Warburg formula, the measured value for A_{260} is marked in the results display and on the results printout with a "◀ ".
Factor	Entry of numbers (five-figure)	(Only when the calculation process "Factor" has been selected) Entering a factor; the number of decimal places determines the number of decimal places in the result.
Corr. with A_{320}	Selection	(Only for nucleic acid methods and for the direct photometric determination of protein) Selection from "Corr. with A_{320} off" and "Corr. with A_{320} on"; "Corr. on" means: the absorbance measured at 320 nm is subtracted from the absorbance results at 260, 280 and 230 nm. Example of application: Correction of turbidity in the sample. When the correction function is switched on, the measuring value for A_{320} is marked with a "◀ " in the results display and on the results printout.
Unit	Selection	The selection from preprogrammed concentration units is method-dependent.
M. unit (molar unit)	Selection	Selection is method-dependent (for nucleic acid measurements only); is required for the conversion of the concentration into molar concentrations (Conversion key).
Cuvette	Selection	Selection from 10 mm, 5 mm, 2 mm and 1 mm optical path length; the result is converted for an optical path length of 10 mm (see Section 12 "Calculation").

The following parameters are offered only when the "Standard" calculation procedure has been programmed:

Parameter	*Entries*	*Explanation*
Std. number	Entry of numbers ("1" to "10")	Number of different standards.
Std. measurement	Selection	Selection from "1x", "2x", "3x" repeat measurement of each standard; a mean value is formed for the further calculation using the repeat measurements.
Regression	Selection	(Only for standard number of at least 4 (for single determination of standards: 5)) Selection from the calculation procedure linear and non-linear regression. For a number of standards greater than 1 and lower than 4 (or 5 respectively), calculation always takes place via linear regression (See Section 12, "Calculation").
Std. 1 to Std. 10	Entry of numbers (five-figure)	Entry of nominal values of standard concentrations; the number of decimal places of the nominal concentration for the first standard determines the number of decimal places in the result.

6.4 Factory-set programmed values

	dsDNA	*ssDNA*	*RNA*	*Oligo*	*Protein*	*Bradford*	*Bradford micro*	*Lowry*	*Lowry micro*	*BCA*	*BCA micro*	*OD 600*
Calculation					Absorbance	Standard	Standard	Standard	Standard	Standard	Standard	
Factor	50.0	37.0	40.0	30.0	- - - - - [1)]	- - - - - [1)]	- - - - - [1)]	- - - - - [1)]	- - - - - [1)]	- - - - - [1)]	- - - - - [1)]	1.000
Corr. with A320	off	off	off	off	off							
Std. number						6	6	6	6	8	5	
Std. measurmnt.					1x[2)]	1x	1x	1x	1x	1x	1x	
Regression						non-linear	non-linear	non-linear	non-linear	non-linear	non-linear	
Unit	µg/mL	µg/mL	µg/mL	µg/µL	µg/mL[3)]	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	
Molar unit	pmol/mL	pmol/mL	pmol/mL	pmol/µL								
Standard 1					- - - - - [4)]	100	1.00	100	1.00	25	0.50	
Standard 2						250	2.5	250	2.5	125	2	
Standard 3						500	5	500	5	250	5	
Standard 4						750	10	750	10	500	10	
Standard 5						1000	15	1000	15	750	20	
Standard 6						1500	25	1500	25	1000		
Standard 7										1500		
Standard 8										2000		
Cuvette	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm	10 mm

Notes:
1) With"Factor" calculation: Input required from user
2) With "Standard" calculation
3) With "Standard" or "Factor" calculation
4) With "Standard" calculation: Input required from user

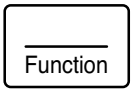
7 Functions

Functions list

Function	***Entries***	***Explanation***
Display results	Call up using [Enter].	Display of the last 100 results (The most-recent result appears first): [▲] [▼]: To select the results. [Enter]: To print out the results that have just been displayed. [Function]: To return to the functions list.
Calibration report	Call up using [Enter].	Printout of the calibrations stored; [▲] [▼]: To select the method. [Enter]: To print out the calibration report. [Function]: To return to the functions list.
Date	Entry of figures	[Enter]: To store.
Time	Entry of figures	[Enter]: To store.
Stored absorbance	Call up using [Enter].	To print out the most-recently measured absorbances (max. 100 measurements). Mean value, standard deviation and CV are calculated and printed out for the values of the most-recently measured method.
Precision measurement	Call up using [Enter].	To perform measurement and precision calculation of ten consecutive measuring values of one sample. For evaluation purposes, the method program of the most-recently selected method method is used.
Photometer test	Call up using [Enter].	To check the photometric accuracy and the wavelength accuracy (see Sec. 13, "Testing the photometer").
Sprache Deutsch Language English Language U.S.English langue française	Selection	Selection of language version; Please note that "English" and "U.S.English" differ due to the format of the date.
Printer DPU 414 Printer serial	Selection	DPU 414: To connect the Eppendorf thermal printer DPU 414 (see Section 4.2, "Printer"). serial: To connect another printer (see Section 4.2, "Printer").
Service		Function is accessible to service technicians only.

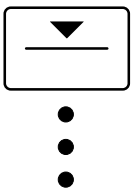
Example: Changing the language version

Call up functions list



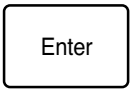

```
FUNKTION      SEITE 1–4

▶ERGEBNISSE ANZEIGEN
 KALIBRATIONS REPORT

 DATUM        27.06.1998
 UHRZEIT          20:44
```

Select desired function



```
FUNKTION      SEITE 3–4

 SPRACHE  DEUTSCH  *
▶LANGUAGE ENGLISH  –
 LANGUAGE U.S.ENGL –
 langue française  –
```

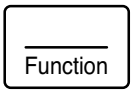
Store function




```
FUNCTION      PAGE 4–4

▶PRINTER DPU 414     *
 PRINTER SERIAL      –

 SERVICE          – – – –

 FUNCTION EXIT
```

To exit the function level, either select the line "FUNCTION EXIT" and press Enter or press the Function key from any line of the functions list. The BioPhotometer then returns to the last method selected.

Exit function level




```
OLIGO

PROGRAMMED FACTOR:
   1  A260 = 20.0     µg/mL

 Blank    or    Sample
```

8 Error messages, result flagging and help texts

Result flagging

Flagging	*Explanation*
1.586 A_{260} ◀	Flagging of A_{260} in the display or on the printout (for method "Protein direct" only): The method was calculated using the Warburg formula.
0.015 A_{320} ◀	Flagging of A_{320} in the display or on the printout (for method "Protein direct" and for nucleic acid methods only): The absorbances at 260, 280 and 230 nm are corrected with the absorbance at 320 nm (see Section 6, "Programming").

Error texts in the results display

Error text	*Explanation / Cause*	*Solution*
+++++	The absorbance measured is greater than 3.0 A.	– Dilute the sample. – Check the cuvette (height of light path must be 8.5 mm). – Clean the cuvette shaft (see Section 9). – Insert the cuvette correctly (the measuring window must be facing the light path). – Use a cuvette made of material that transmits light at the measuring wavelengths used (e.g. quartz glass or UVette® from Eppendorf for nucleic acid measurement).
! ! ! ! !	The calculated result cannot be displayed (value too high).	Check the parameter (Is the factor too high?).
– – – – –	(Instead of a value for the ratio:) Ratio cannot be calculated because one of the absorbance values used for calculating the ratio is 0 A or > 3.0 A.	Repeat the measurement (dilute sample if necessary).

8 Error messages, result flagging and help texts

Error texts in measuring procedure

Error text	*Explanation / Cause*	*Solution*
Measure blank first	No blank has been measured for the method selected.	Measure the blank.
Measure standard first	No valid calibration for the method selected.	– Measure standards. – Program a different calculation (fixed factor or direct absorbance measurement).
not within calibration	(For calculation via non-linear regression only:) The sample result is not within the calibration range.	Repeat the measurement (dilute sample if necessary).
Measurement module Error 1 Measurement module Error 2 Measurement module Error 3	Different errors in measurement module.	Contact Service.

Error texts in calibration procedure

Error text	*Explanation / Cause*	*Solution*
No STD method	The Standard measuring key was pressed although "Standard" was not programmed as a procedure for the method selected.	– Re-measure the methods without standard request. – Program the "standard" calculation.
Measured values not plausible	(For one-point calibration:) Absorbance measured is 0 A.	Re-measure standard. (Prepare again if necessary).
Measured values not monotonous	(For multiple-point calibration:) The measured values do not produce monotonously rising or falling sequences.	Check standards and re-measure in the correct sequence (ascending concentration).
Calibration curve is not monotonous	(For non-linear regression:) The calculated curve is not monotonous.	Check standards and re-measure in the correct sequence (ascending concentration).

8 Error messages, result flagging and help texts

Error text	*Explanation / Cause*	*Solution*
CV greater than 10 %	(Following standard measurements:) The scattering of the measured values around the calculated calibration line or curve is very large (see Section 12, "Calculation").	Check calibration result. – Enter : Store calibration. – Clear : Abort calibration. Recalibrate or use the calibration stored.

Error texts in programming procedure

Error text	*Explanation / Cause*	*Solution*
Method parameter incorrect. Please check	Method parameters incorrectly entered.	Check parameters and re-enter them if necessary.
Please program standards ascending	(For multiple-point calibration:) Standard nominal values have not been programmed in ascending order.	Check programming and enter nominal values in ascending order.

Other error texts

Error text	*Explanation / Cause*	*Solution*
Entry invalid	(When a serial sample number is entered via the ___ Sample No. key:) A number outside of the range 1 to 999 has been entered.	Enter a number within the specified range.

Help texts

Error text	*Explanation / Cause*	*Solution*
Please program standard	(In the display after method selection:) For the method selected, the calculation "Standard" has been programmed; but the nominal concentrations for the standards have not yet been programmed.	– Program nominal concentrations for the standards (Parameter key). – Program another calculation without standards.
Please program factor	(In the display after method selection:) For the method selected, the calculation "Factor" has been programmed; but the value for the factor has not yet been programmed.	– Program the value for the factor (Parameter key). – Program another calculation.

9 Maintenance and cleaning

Photometer

– Disconnect the device from the main power source before carrying out maintenance work or to change the fuses.
The inside of the device is a high-voltage area. Danger!

– Wipe the entire device using a moist cloth and a mild cleaning agent.

– Disinfect the device using a lightly moistened cloth and a 70 % ethanol/water mixture.

– Do not allow any liquid to enter the device.

Cuvette shaft

– Clean the cuvette shaft using a moist cotton swab only. Do not use large quantities of liquid (e.g. spray bottles).

– When the device is not being used, protect the cuvette shaft from dust using the seal provided.
Dust or residue from the measuring solutions in the optical light path can cause inaccurate measurements.

Changing the fuses

– Disconnect the device from the mains supply.

– The fuse holder is located above the mains connection (see picture in Sec. 4.1). The holder is held in position by a small elastic stop lever on its underside.

– Push the stop lever upwards and pull out the holder.

– Change the fuses (for specifications, see Sec. 2, “Technical data“).

– Press the holder into the attachment until the stop lever clicks into place.

– Plug the device into the mains supply.

10 Short instructions

Preparation

The BioPhotometer is ready to measure immediately after being switched on.

Methods

7□ dsDNA | 8□ ssDNA | 9□ RNA
4□ Protein | 5□ OD 600 | 6□ Oligo
1□ Bradford | 2□ Lowry | 3□ BCA

7□ dsDNA | 8□ ssDNA | 9□ RNA | 6□ Oligo

dsDNA ssDNA RNA Oligo

- Direct measurement of the nucleic acids at 260 nm.
- Ratios A260/A280 and A260/A230.
- Optional correction of absorbance values via A320.
- Measurement using quartz-glass cuvette or UVette® from Eppendorf.

5□ OD 600

OD 600

- Direct measurement of the density of bacteria suspensions at 600 nm (turbidity measurement).
- Measurement using glass cuvette or plastic cuvette.

4□ Protein

Protein

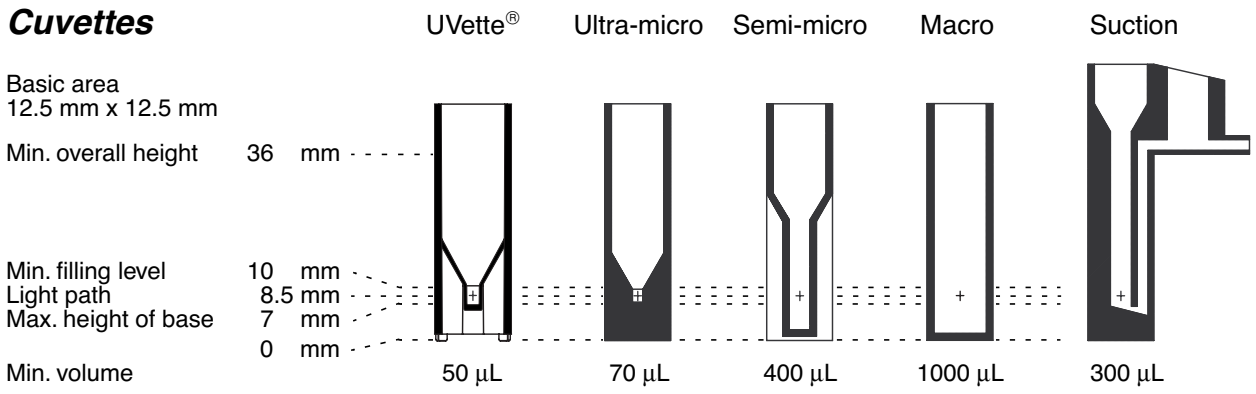
- Direct measurement of protein at 280 nm.
- Direct measurement of the absorbance, or calculation via factor, standard or Warburg formula.
- Optional correction of absorbance values via A320.
- Measurement using quartz-glass cuvette or UVette® from Eppendorf.

1□ Bradford | 2□ Lowry | 3□ BCA

Bradford Lowry BCA
Bradford micro Lowry micro BCA micro

- Measurement of protein using Bradford-, Lowry- or BCA reagent.
- Direct measurement of the absorbance, or calculation via factor or calibration (single-point calibration, linear regression or non-linear regression).
- Number and nominal values of the calibrators are programmable.
- The protein methods are also available on a micro-scale (Press the Method key twice).
- Measurement using glass cuvette or plastic cuvette.

Cuvettes



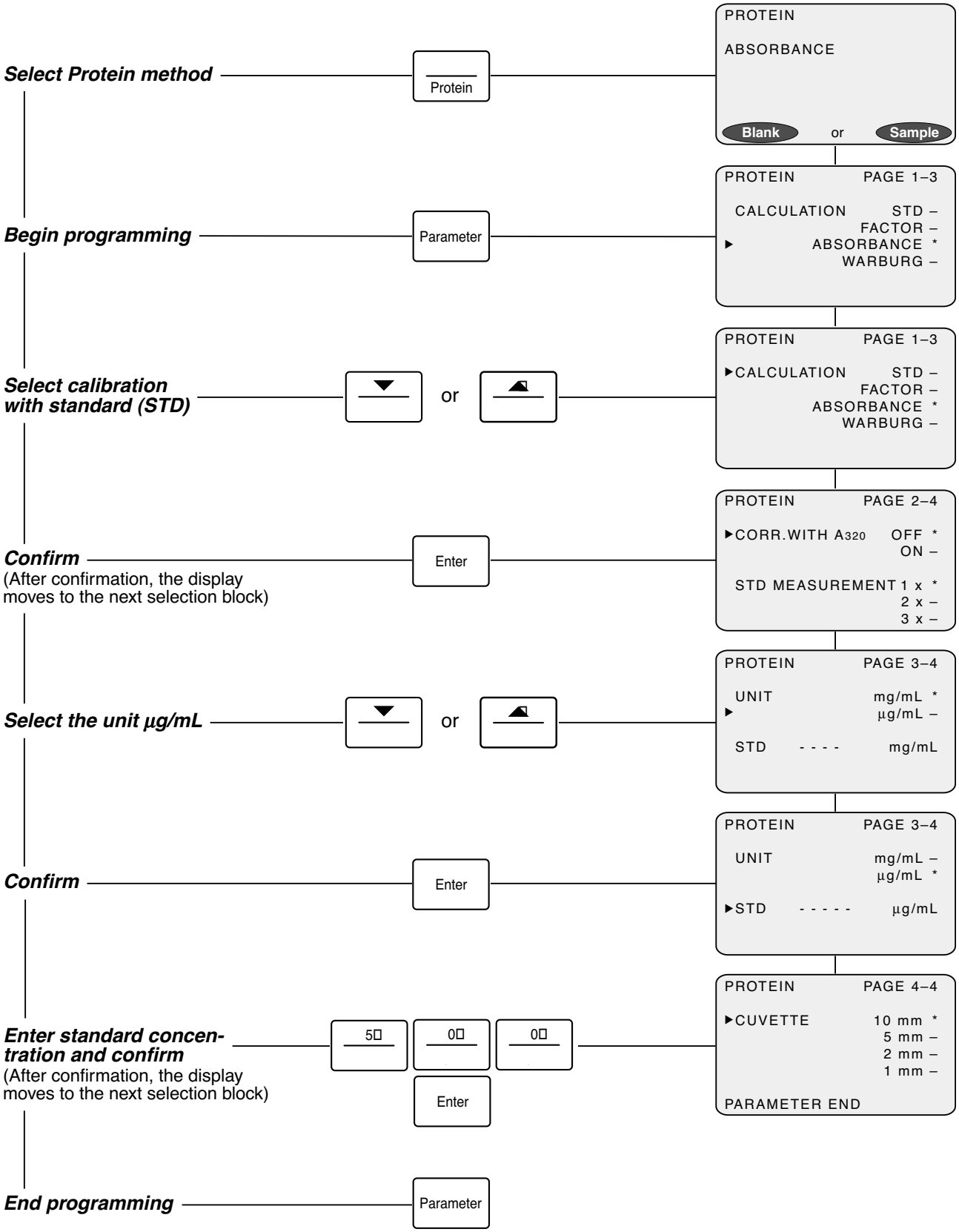
10 Short instructions

Programming

The factory-set method programs may be changed as required.

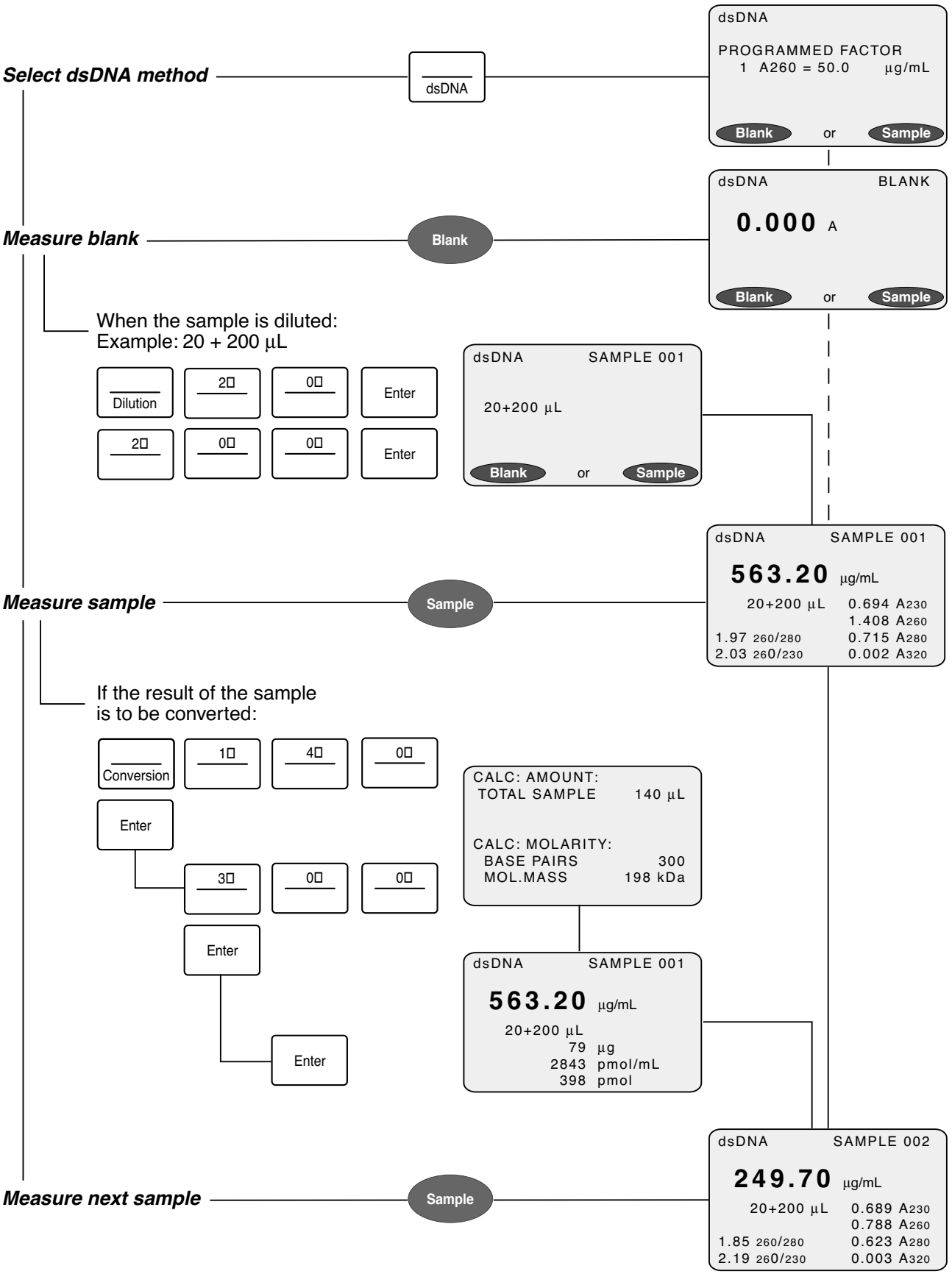
Example:

Programming of the unit "µg/mL" and of calculation via standard (500 µg/mL) for the Protein method.



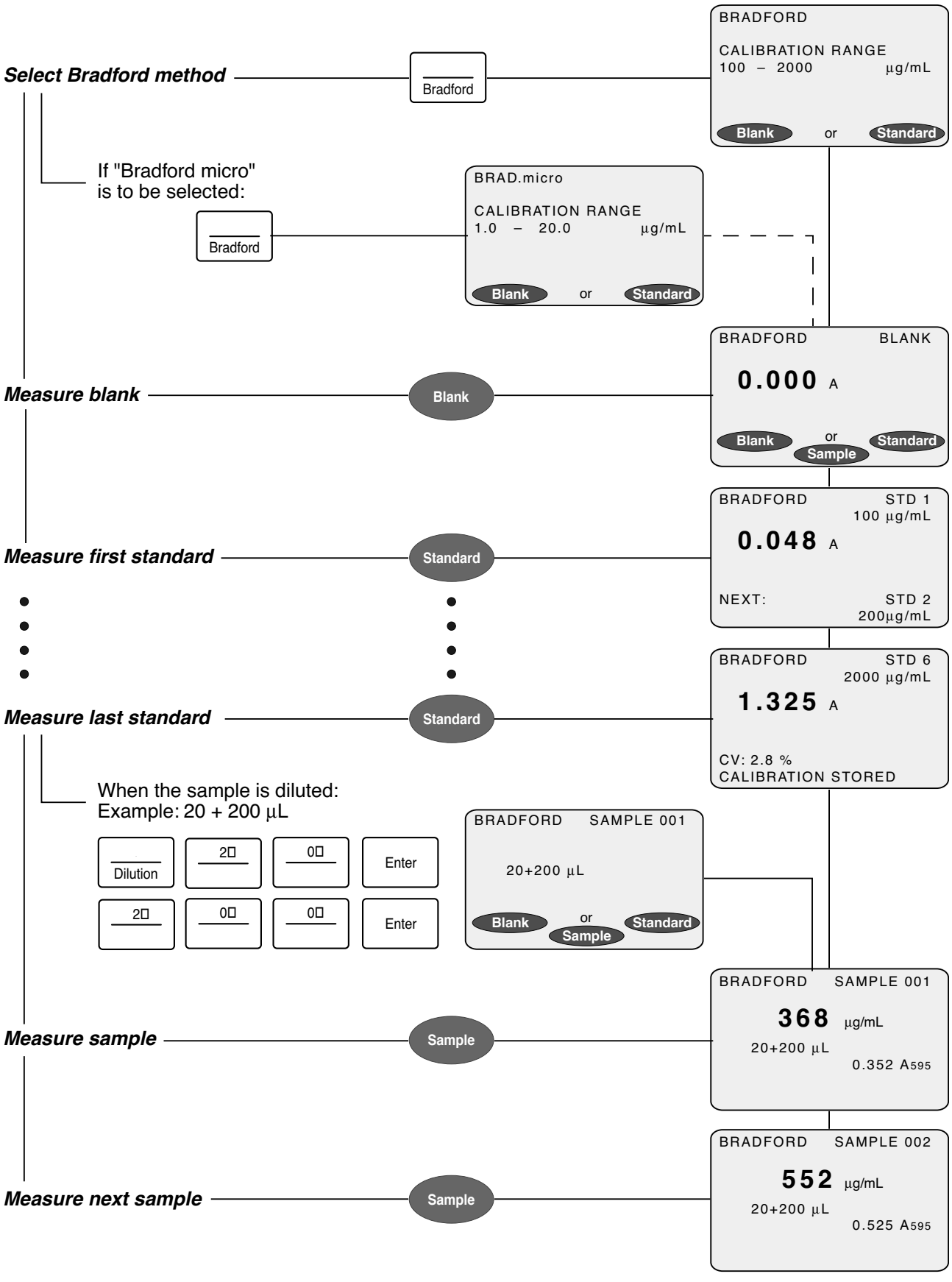
10 Short instructions

Measuring procedure for dsDNA



10 Short instructions

Bradford measuring procedure



11 Ordering information

11.1 Ordering information

Order no.	
	Photometer
6131 000.012	BioPhotometer (230 V; 50/60 Hz; European plug) (additional power supply variants available)
6131 810.006	BioPhotometer Software Package for online data transfer on PC
6131 928.007	Secondary UV-VIS filter, Test Filter Set, for checking the BioPhotometer
	Printer
6131 011.006	Thermal Printer DPU 414, incl. power supply 230 V unit and printer cable
0013 021.566	Thermal paper (5 rolls)
	UVette® ***(Disposable plastic cuvette for the UV / VIS range, 220 to 1,600 nm)***
0030 106.300	UVette®, 80 pcs., individually packaged
4308 078.006	Cuvette stand for 16 cuvettes

11.2 Ordering information for North America

Order no.	
	Photometer
952 00 000-4	BioPhotometer
952 01 020-4	BioPhotometer Software Package, for online data transfer to PC
952 01 022-1	Secondary UV-VIS Filter Test Set, for verifying photometric precision and the wavelength accuracy (NIST Traceable)
	Printer
952 01 015-8	Thermal Printer DPU-414, requires power supply unit and printer cable
952 01 017-4	Power Supply Unit for Thermal Printer DPU-414, 115 V
952 01 016-6	Power Supply Unit for Thermal Printer DPU-414, 230 V
952 01 018-2	Printer Cable, for connecting serial printer
952 01 040-9	Printer Paper, 5 rolls
	UVette® *
952 01 005-1	UVette®, 80 original Eppendorf disposable, individually packaged cuvettes
940 00 110-2	Cuvette Stand

* U.S. Patent No. 6,249,345

12 Calculation

12.1 Nucleic acids (dsDNA, ssDNA, RNA, oligo)

Calculation via factor

$C = A_{260} \times F$

C = Calculated concentration
A_{260} = Absorbance measured at 260 nm
F = Factor (method-specific programming using the [Parameter] key)

The nucleic acid methods have the following special feature: The programmed factor is always based on the unit of concentration "µg/mL". If the unit of concentration "µg/µL" is selected, the factor is converted internally:

F' = F / 1000
F' = Converted factor; used for the calculation of the concentration.

Sample dilution

$C_{Dil,\ corr} = C \times (V_P + V_{Dil}) / V_P$

$C_{Dil,\ corr}$ = Result converted using dilution factor
V_P = Volume of the sample in the measuring solution (entered using the [Dilution] key)
V_{Dil} = Volume of the diluent in the measuring solution (entered using the [Dilution] key)

Optical path length of the cuvette

Application: Using cuvettes with an optical path length of 1 mm, 2 mm or 5 mm.

The optical path length of the cuvette can be programmed for each method using the [Parameter] key.

$A_{cuv,\ corr} = A \times 2$ (with an optical path length of 5 mm)
$A_{cuv,\ corr} = A \times 5$ (with an optical path length of 2 mm)
$A_{cuv,\ corr} = A \times 10$ (with an optical path length of 1 mm)
$A_{cuv,\ corr}$ = Absorbance converted in accordance with an optical path length of 10 mm

Correction A_{320}

Application: Partial correction of incorrect absorbance caused by turbidity in the measuring solution.

The calculation procedure with or without correction A_{320} can be programmed for each method using the [Parameter] key.

$A_{x,\ corr} = A_x - A_{320}$

$A_{x,\ corr}$ = Absorbance at wavelength of 230, 260 and 280 nm, corrected mathematically
A_x = Absorbance measured at wavelength of 230, 260 and 280 nm
A_{320} = Absorbance measured at wavelength of 320 nm

The corrected absorbance is used for further calculation of results.

Conversion key: Calculating the quantity

Application: Calculating the quantity of nucleic acid in the total sample volume.

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

M = Calculated overall quantity of nucleic acid in sample vessel
C = Calculated concentration
$V_{P,\ total}$ = Volume of the sample in the sample vessel (entered using the [Conversion] key)

12 Calculation

Conversion key: Calculating the molar concentration

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

$C_{mol} = C / N$

C_{mol} = Molar concentration (calculated)
N = Relative molar mass, in kDa (entered using the Conversion key)

If, instead of the relative molar mass, the number of bases / base pairs per molecule has been entered, N is calculated using the number of bases / base pairs:

dsDNA: $N = bp \times 2 \times 330 \times 10^{-3}$
ssDNA, RNA, Oligo: $N = b \times 330 \times 10^{-3}$
N = Calculated relative molar mass, in kDa
bp = Number of base pairs per molecule (dsDNA)
b = Number of bases per molecule (ssDNA, RNA, Oligo)

The unit for molar concentration is programmed for each method using the Parameter key.

12.2 Direct photometric determination of protein

Selection for calculation of results:

- Absorbance
- Calculation of the concentration via factor
- Calculation of the concentration via one-point calibration
- Calculation of the concentration via Warburg formula

Calculation of the concentration via factor

See Section 12.1; Measuring wavelength: 280 nm

When the factor is entered using the Parameter key, the unit of concentration which has been programmed must be taken into consideration.

Calculation of the concentration via standard (one-point calibration)

$F = C_S / A_S$
F = Calculated factor
C_S = Nominal concentration of the standard (method-specific programming using the Parameter key)
A_S = Measured absorbance of the standard

If the standard multiple measurement (2x, 3x) has been programmed, calculation is based on the absorbances measured, including the zero value, via linear regression. After the regression has been calculated, a CV (coefficient of variation in "%") value is formed as a measure of the scattering of the measured values. If the CV value is greater than 10 %, it appears in the display. In this case, the calibration is not stored automatically; it must first be confirmed by the user (see Section 12.3).

The calculation of the sample concentration is carried out using the calculated factor:

$C = A_{280} \times F$

Calculation of the concentration via Warburg formula

$C = 1.55 \times A_{280} - 0.76 \times A_{260}$ for "mg/mL" concentration unit
$C = (1.55 \times E_{280} - 0.76 \times A_{260}) \times 1000$ for "µg/mL" concentration unit

Sample dilution, optical light path of the cuvette and correction A_{320}

See Section 12.1.

12.3 Protein with addition of reagent

Methods: Bradford, Bradford micro, BCA, BCA micro, Lowry, Lowry micro

Selection for the calculation of results:

- Absorbance
- Calculation of the concentration via factor
- Calculation of the concentration via standard

Selection for the calculation procedures via standard:

- One-point calibration
- Multiple-point calibration (standard line)
- Multiple-point calibration (standard curve)

Calculating the concentration via factor and calculating the concentration via standard (one-point calibration)

See Section 12.2; Measuring wavelength: 595 nm (Bradford; Lowry) or 562 nm (BCA)

Calculating the concentration via standard (multiple-point calibration; calibration line)

A calibration line (concentration as a function of the absorbance) is calculated from 2 to 10 standards, which are measured in single, double or triple determination. The equation of the line is calculated via linear regression.

$C = a_o + a_1 A$

a_1 = Slope of the calibration line (Factor)

a_o = Intersection point of the calibration lines with the concentration axis (concentration of a sample with the absorbance "0" [Offset])

After the calibration has been calculated, the CV value (coefficient of variation in "%") is calculated (exception: two-point calibration with single determination of the two standards). The CV value is a measure for the scattering of the measured values around the calculated calibration line. If the value is greater than 10 %, the calibration is not stored automatically; it must first be confirmed by the user. In the case of more than two standards, the CV value always appears in the display (even when the value is lower than 10 %).

The calculated parameters ("a_o" and "a_1") of the stored calibration line can be printed out by calling up the functions list by pressing the Function key.

Calculating the concentration via standard (multiple-point calibration; calibration curve)

A calibration curve (concentration as function of the absorbance) is calculated from 5 to 10 standards measured in single determination or from 4 to 10 standards measured in double or triple determination. The non-linear regression is calculated via a third-grade polynomial.

$C = a_o + a_1 A + a_2 A^2 + a_3 A^3 + \ldots$

a = Coefficients (The coefficients are determined using the least square method).

CV value: see above (linear regression).

The calculated parameters of the stored calibration line can be printed via the Function key.

12 Calculation

Sample dilution and optical light path of the cuvette

See Section 12.1.

12.4 OD 600

The measured values appear as absorbance values measured at a wavelength of 595 nm.

Sample dilution and optical light path of the cuvette

See Section 12.1.

13 Testing the photometer

To enable the photometric accuracy and the wavelength accuracy to be tested, a filter set (secondary UV-VIS filter) is available from Eppendorf. This set contains three filters ("Sample A_1", "Sample A_2" and "Sample A_3") for testing the photometric accuracy and two filters ("Sample 260 nm" and "Sample 280 nm") for testing the wavelength accuracy. The absorbance of the filters is measured against a blank filter ("Blank A_0").

To carry out these measurements, blank filters and "sample filters" (test filters) are inserted into the cuvette holder in the same manner as cuvettes. When doing so, please ensure that the label with the filter description is facing the user. The absorbance values measured for the test filters are compared to those within the range of permitted values. The limits for the permitted range are contained in a table found on the inside of the lid of the filter box (see Figure: "X.XXX – X.XXX A").

BioPhotometer — **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 / Systematische Messabweichung					
		of wavelength / der Wellenlänge		of photometer / des Photometers			
		Traceable to / rückführbar auf					
		Nist: SRM 2034, SN: 99		Nist: SRM 2031a, SN: 577			
	(A) / (E)	Limiting values (A) / Grenzwerte (E)					
230 nm	0.000			X.XXX - X.XXX	X.XXX - X.XXX	X.XXX - X.XXX	empty / leer
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	
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	
		Random error / Zufällige Messabweichung					
		of wavelength / der Wellenlänge		of photometer / des Photometers			
230 – 595 nm		Limiting values CV (%) / Grenzwerte VK (%)					
	0.000	≤ 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

Fig.: Inside of the lid of the filter box

13 Testing the photometer

Test procedure

- Carry out the test at approximately 20 °C.
- Remove the filter from the filter box for a brief period only. Make sure that the surface of the filter is not contaminated or damaged.
- Protect the filter from dust, heat, liquid and aggressive vapors.
- When inserting the filter, ensure that the label with the filter description is facing the user.
- Select the function "Photometer test".
  This function is contained in devices with a software version of V 1.20 onwards. Contact Eppendorf before using the test filter with an older software version.
- Select the test filter.
  - "A_1", "A_2" or "A_3" for the measurement of the photometric accuracy at 230, 260, 280, 320, 562 and 595 nm.
  - "A_{260}" or "A_{280}" for the measurement of the wavelength accuracy at 260 or 280 nm.
- Follow the instructions in the photometer display for the measurement of "Blank" and "Sample".
  The device carries out 10 measuring cycles and then prints out the mean values for the absorbances at the respective wavelengths.
- Compare the absorbance values with the permitted value range.
- In addition to information on accuracy values, the printout contains details of precision as well. The mean value and the CV are calculated from each of the ten measurements.

If the absorbances measured are not within the permitted value range, please contact the Service Department at Eppendorf. The filters should be recalibrated by Eppendorf after two years.

Conformity Declaration for BioPhotometer 6131
in accordance with enclosure 15 of "Eichordnung" (German standardization regulations)

Description of measurement

Device used:	Single-beam filter photometer with reference beam and fixed wavelengths
Type:	BioPhotometer 6131
Manufacturer / Distributor:	Eppendorf AG, Hamburg
Mode of instruction:	Operating manual

1. Measuring system

Light path:	Lamp > aperture > lens > aperture > cuvette > aperture > diffraction grating > aperture > photodiode
Light source:	Xenon flash lamp Continuum spectral range 220 to 2,000 nm
Spectral apparatus:	Grating polychromator
Radiation receiver:	Silicon photodiode Spectral range 200 to 1,100 nm
Cuvette:	Quartz glass, optical special glass or plastic, depending on measuring wavelength
Cuvette types:	10 mm macro min. vol. 1000 µl 10 mm semi-micro min. vol. 400 µl 10 mm suction min. vol. 300 µl 10 mm ultra-micro min. vol. 70 µl
Cuvette temperature:	Not available
Results display:	Illuminated, graphic LCD, 33 x 66 mm^2
Measured values displayed:	Absorption, mass concentration, molar concentration

2. Measuring procedures

Determination of the cuvette blank:	Wavelength-dependent individual measured value of the cuvette used
Concentration determination:	Lambert-Beer-Bourguer law
Reference measurement on reference material:	Check with calibrated secondary standards

3. Measuring range of the spectral absorption rate

0.000 to 3.000 A
The error limits listed can be exceeded outside these measuring ranges as well as with nominal conditions of use other than those listed below.

4. Nominal conditions of use

Cuvette blank:	Depending on cuvette used
Wavelengths:	Xenon 230, 260, 280, 320, 562, 595 nm
Warm-up time:	None
Supply voltage:	100 to 240 V ± 10 %, 50 to 60 Hz ± 5 %
Ambient temperature:	15 to 35 °C
Relative humidity:	15 to 70 %

5. Error limits and other limiting values

Relative photometric uncertainty of the spectral absorption rate with all wavelengths for an individual measurement:	± 1.5 % at 1 A
Relative photometric short-time standard deviation:	≤ 0.5 % at 1 A
Wavelength systematic error:	± 1 nm at 230 to 280 nm, ± 2 nm at 320 to 595 nm
Spectral half-intensity width:	≤ 5 nm at 230 to 320 nm, ≤ 7 nm at 562 and 595 nm
Integral fault-radiation level:	≤ 0.03 % at 260 nm with GG 375-3 (Schott)

Date: 25.09.2000
Eppendorf AG
Quality and standards

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 6131

Produkttyp, Product type:

Photometer

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

73/23/EWG, EN 61010-1

89/336/EWG, EN 55011/B, EN 61000-6-1, EN 61000-3-2, EN 61000-3-3

Vorstand, Board of Management:

Projektmanagement, Project Management:

07.08.2003

Hamburg, Date:

ISO CERTIFIED 9001

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