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Evaluating the functionality of Eppendorf BioPhotometer® and Eppendorf BioSpectrometer® using a Secondary UV-VIS Filter Set

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Introduction

The quality of a reading displayed after a measurement of a sample can only be as good as the measurement itself. It is therefore important to control potential sources of error that could negatively influence the measurement, or at least to keep them within clearly defined limits. Typically, it can clearly be differentiated between technical errors and errors resulting from incorrect handling. In the case of photometric quantification, the latter can include too low or too high sample concentration, inadequate sample mixing or an incorrectly programmed method factor.

In contrast to the handling errors, which are relatively easy to discover and correct, technical errors can only be determined through comparative measurements with reference materials, and generally require an adjustment of the respective device in order to correct them.

For each device produced by Eppendorf clearly defined limits are given within which technical errors do not negatively change the accuracy of the device. These limits contain the systematic errors of the device, the measurement and the calibration medium.

Regular inspection of the devices is required in order to ensure the observance of these limits during use. Labs working on the basis of a quality management system (e.g. ISO 9001, ISO 17025, GLP, GMP) have pledged themselves to this regular verification of their measuring equipment.

Eppendorf offers test filter sets as certified reference material for comparative measurements. These filter sets are traceable to reference standards of the National Institute of Standards and Technology (NIST®, Gaithersburg, MD, USA). They serve to test the photometric and wavelength accuracy of Eppendorf Photometers. By comparing the measured values with the limiting values of each filter it is possible to assess for the trueness and precision of the photometer.
The detection ranges of each test filter set are validated with a typical reference photometer. The filters are available for the BioPhotometer plus, BioPhotometer D30, BioSpectrometer basic, BioSpectrometer kinetic and the BioSpectrometer fluorescence. The BioSpectrometer basic and BioSpectrometer kinetic share one common test filter set.

Theoretical background

The meanings of the terms used in connection with the testing of devices are more precisely defined in the norm ISO 3534-1. As many of these terms used in the literature are often used in an ambiguous manner, figure 1 explains the connections between some of the fundamental terms. Besides errors resulting from incorrect handling, one differentiates between two special types of technical errors having a significant influence on the measuring result: systematic and random errors. "Systematic errors" caused by interfering influences (e.g. dirty light path) or defective parts (e.g. falsely adjusted optics) can result in a deviation of the measurement result from the real value. These errors can only be discovered through comparative measurements with reference materials (e.g. Secondary UV-VIS-Filter) (Fig. 1). Therefore, a multiple determination is carried out with the reference material, and the mean value resulting from the measurement series is compared with the correct value. The mathematical difference between mean value and correct value is the quantitative description of the systematic error. Depending upon whether the systematic error lies within previously defined limiting values, one can then make a qualitative statement of trueness as to whether the device is functioning correctly or not. The second type of error is designated "random error", whose cause can often not be characterized and rarely be avoided. Consequently, random errors influence the spread of the measured values and thus, the precision of the measuring device (Fig. 1). The connection between trueness and precision with respect to exact and reproducible measurements is graphically explained in figure 2.

In figure 2A, all measured values, and thus also their mean value, lie within the defined limits and indicate only minor scattering (random error). Assuming that the random error also lies within the defined limits (data not shown), this measuring device is delivering exact, reproducible measurement data with a high degree of trueness and precision. In comparison to this, the measurement series in 2B is based on a systematic error. As a result, all measured values have been deferred to the same extent and lie outside of the given limits. Interestingly, the individual measured values vary from one another only very little as shown in Fig. 2A, indicating that the device is delivering precise but false measurement results. With a Eppendorf BioPhotometer or BioSpectrometer, such a measurement result might be caused by, for example, a dirty light path. In Fig. 2C, the individual measured values vary strongly from one another, indicating a pronounced random error. It is impossible to receive consistent measured values with such a device. The distribution of the measured values in figure 2D shows high systematic and random errors. The device demonstrates both poor trueness and poor precision. Exact and reproducible measurements with a high degree of accuracy can only be obtained with a device for which both the trueness and the precision lie within the defined limiting values. If the results of the Photometer test described in the short instructions herein look like the pattern in figures 2B – D the BioPhotometer or BioSpectrometer should be checked by the technical service to perform according to the specifications.

Kind of error →	**Systematic error, bias** They result e.g. from falsely adjusted optics or a dirty light path.	**Random error** The origin of random errors can often not be characterized.
Quantitative value →	**Systematic error, bias** Mathematical difference between the mean value of a multiple determination and the correct value.	**Random error** Closeness of agreement between the independent measurement values of a multiple determination that is normally expressed in terms of standard deviation or coefficient of variation.
Qualitative criteria →	**Trueness** **(obsolete: "accuracy of the mean")** Trueness considers the agreement between the average value of multiple determination in relation to the correct value. Depending on the systematic error in relation to the defined limiting values for a device the term trueness gives a qualitative expression about the correct function of a device with respect to the systematic error. (The device is working true/untrue).	**Precision** Precision is a qualitative term for the agreement between different values of a set of measurements. Depending on the statistical spread of the measurement values in relation to the defined limiting values of a device it is possible to conclude for the correct function of a device with respect to the random error. (The device is working precise/imprecise).

affect

Accuracy
Accuracy is a qualitative expression for the agreement between a single measurement value and the correct value.
As accuracy considers only one single value the term accuracy includes both the systematic and random error and as to that, it contains information about the trueness and precision of a device.

Figure 1: Explanation of the terms trueness, precision and accuracy according to the stipulations of ISO 3534-1. Conceptually, it can be differentiated between the type of error, the mathematically quantified dimensions of the error and the qualitative description of the function of a device. In contrast to trueness and precision, the term accuracy is hardly used when testing the functionality of a device and is only listed for the sake of completeness.

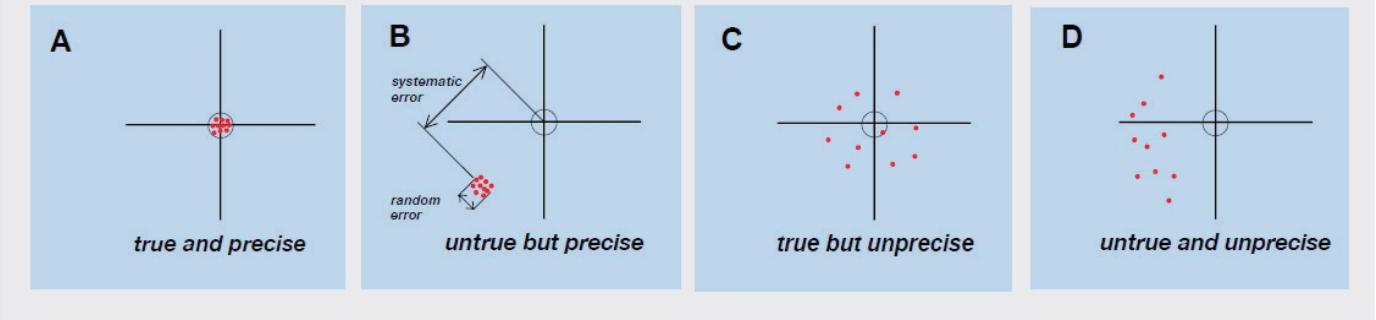


Figure 2: Graphic representation of the connection between trueness and precision when testing measuring devices. Individual measured values are shown as points. The middle of the cross of the axes represents the correct value, while the circle represents the permitted limits for the systematic error. In Fig. B, the deviations representing the systematic and random error are also shown for additional clarity.

Short instructions for the photometer test on the Eppendorf BioPhotometer® and Eppendorf BioSpectrometer®

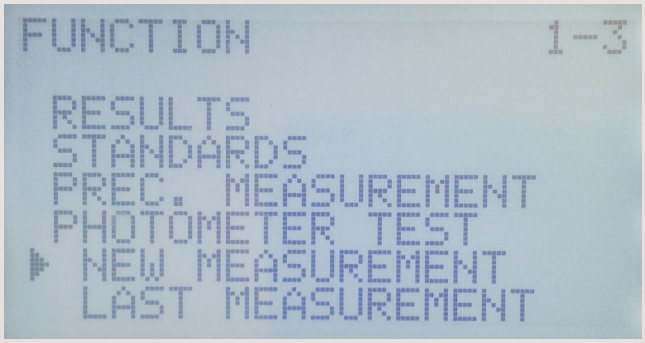
A) General information:

> Measurements should be carried out at a temperature of approx. 20 °C
> In order to carry out the photometer test, the Secondary UV-VIS-Filter Set is required. These filters are inserted into the cuvette shaft. For the test the specific test filter sets for the BioPhotometer and BioSpectrometer are required (s. ordering information).
> Test filters need to be clean and free of dust. The stickers need to face the user.
> To simplify documentation of the measured values, we recommend connecting the device to a printer.
> Use the forms provided for each photometer (s. appendix) to transfer the limits given in the certificate in the lid of the filter box and to enter the measured values of the photometer test.

B) Measurement instructions for the BioPhotometer plus

> Switch on the BioPhotometer plus.
> Press the FUNCTION key and select the NEW MEASUREMENT function under PHOTOMETER TEST in the menu using the cursor keys (▼▲) (Fig. 3A). Confirm with ENTER.
> The following measurement protocols appear: SAMPLE 260 nm and SAMPLE 280 nm for determination of trueness and precision of wavelengths 260 and 280 nm as well as SAMPLE A1, SAMPLE A2 and SAMPLE A3 for determination of metric trueness and precision at 230, 260, 280, 340, 405, 490, 550, 595 and 650 nm.
> Select the appropriate measurement protocol with the cursor keys and confirm the selection with ENTER (Fig. 3B). (We recommend to carry out all 5 measurement protocols when testing the BioPhotometer plus)
> Insert the BLANK A0 filter.
> Press ENTER to carry out the zero value adjustment.
> Insert the appropriate SAMPLE filter.
> Press ENTER. The BioPhotometer plus automatically carries out 15 measuring cycles and calculates mean values and coefficients of variation (CV).
> Compare the measured values and CVs with the limiting values permitted for the relevant test filter. The limiting values are found on the certificate in the lid of the filter box.
> Press ENTER or FUNCTION in order to select the next measurement protocol.

A



B

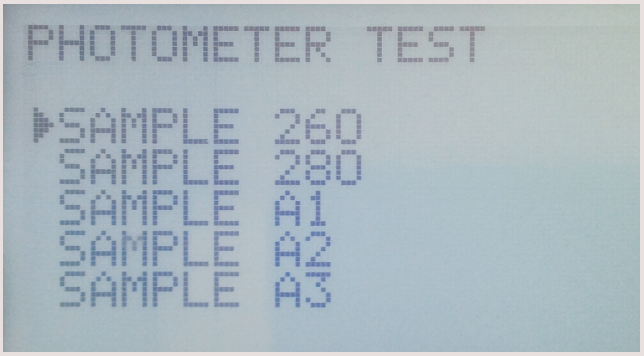


Figure 3
A: Function overview BioPhotometer plus
B: Selection overview of the Photometer Test - BioPhotometer plus

C) Measuring instructions for the BioPhotometer D30 and BioSpectrometer

> Switch on the device.
> If the main menu appears, press the function button on the device or press the „softkey“ function that is located in the lower right corner of the display (Fig. 4A).
> Go here in the „Subgroup“, „Device calibration“ and select here „Photometer unit” (BioPhotometer D30) or “Spectrometer unit“ (BioSpectrometer) (Fig. 4B). Open the method with ENTER.
> Two measuring protocols are now selectable (Fig. 4C):
a) Check wavelength accuracy: Detection of precision and trueness at the wavelength 260, 280 and 800 nm. For BioPhotometer D30 only the wavelengths 260 and 280 nm are checked.
b) Check photometric accuracy: With the Filter SAMPLE A1, SAMPLE A2 and SAMPLE A3 the photometric accuracy and precision is checked at 260, 280, 320, 405, 550, 562, 595, 700 und 800 nm (BioPhotometer D30: 260, 280, 320, 405, 550, 562, 595 nm).
> Select with the cursor keys the desired measuring protocol and confirm with ENTER.
> Insert the Filter A0 for the Blank measurement.
> Press „Blank“ key on the device.
> Insert the correct SAMPLE Filter into the cuvette shaft.
> Press „Sample“. The BioPhotometer D30 and the Bio-Spectrometer carry out 15 measuring cycles and calculate the average values and the coefficient of variation (CV).
> Compare the average and CV values with the values stated on the certificate of the test filter set. The certificate is located on the lid of the filter box.
> With the BioSpectrometer fluorescence additionally the fluorescence unit can be checked. For fluorescence measurements two dynamic measuring ranges can be applied depending on the fluorescensce intensity of the sample. These dynamic ranges are related with respect to the measuring intensity in a defined ratio. With the reference filter (F1) for the fluorescence unit this ratio can be checked (Fig. 4D).

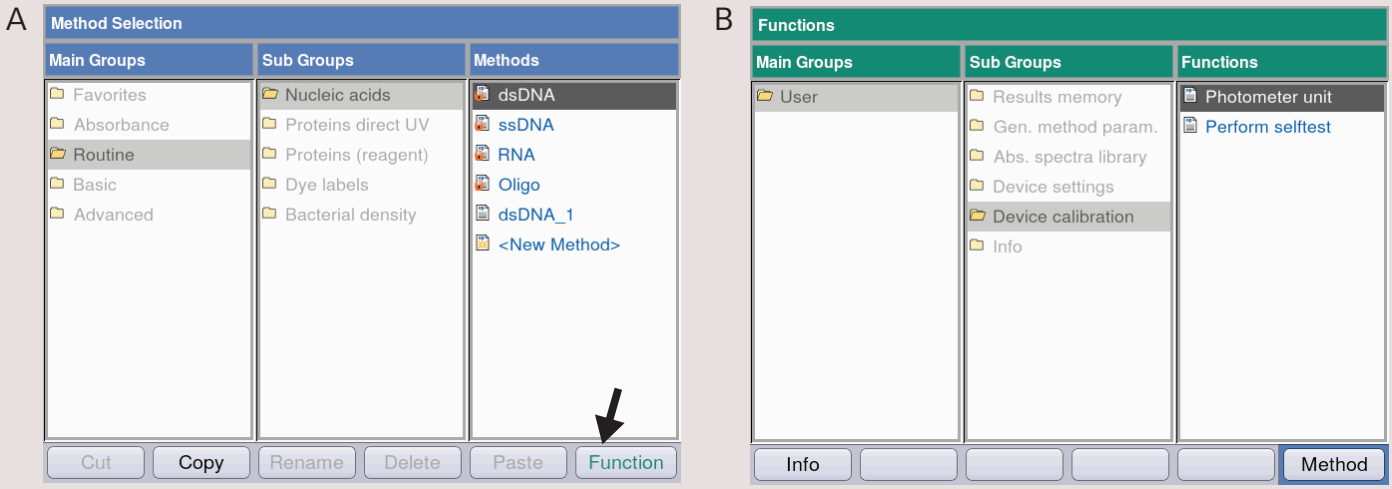


Figure 4
A) Main Menu of the BioSpectrometer and BioPhotometer D30. By using the softkey „Function“ (Arrow) you can enter the device functions incl. Photometer or Spectrometertest
B) Functions overview of the BioSpectrometer and BioPhotometer D30: The photometer or spectrometertest is located in „Sub Group“ /„Device calibration“

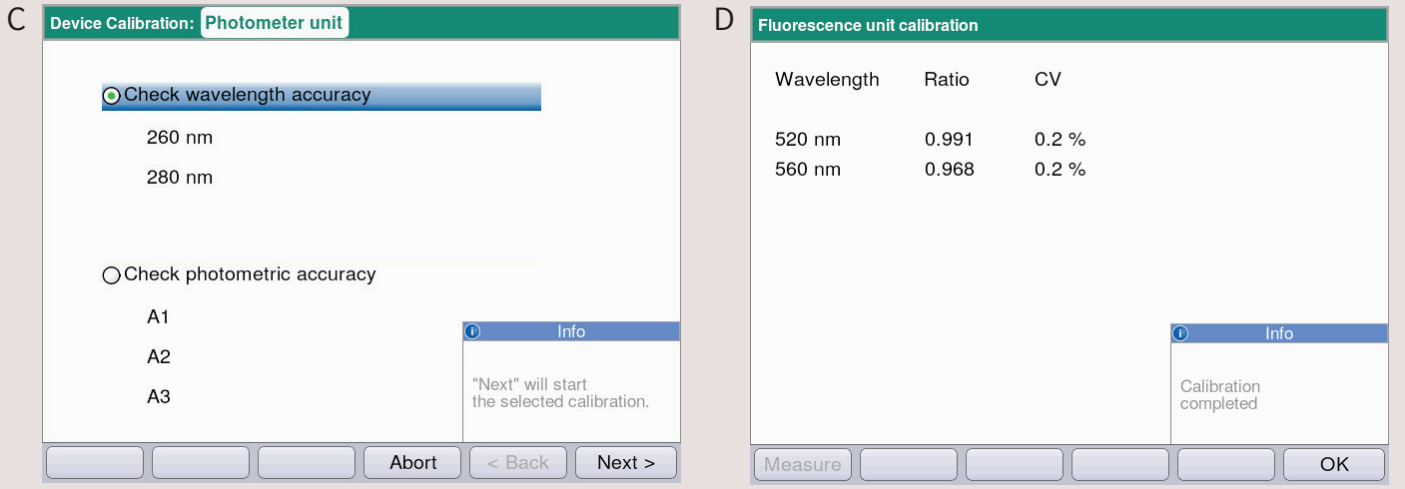


Figure 4
C) Measuring protocols to determine the wavelength and photometric accuracy of the BioPhotometer and BioSpectrometer
D) Checking the fluorescence unit on the BioSpectrometer fluorescence

D) Troubleshooting

When the measured values do not fall within the given range of limits after a repeat measurement, have your BioPhotometer or BioSpectrometer tested by a trained service technician. The certified limits of the Secondary UV-VIS-Filter are valid for a maximum of 2 years. A recalibration of the Secondary UV-VIS-Filter has to be carried out by Technical Service. Please contact your local Eppendorf sales organization for further details concerning technical support.

E) Definitions

Photometric Accuracy: is the maximum deviation of the average values from the values displayed by the photometer in a specific measuring range

Wavelength accuracy: is the maximum deviation of the average values from the values displayed by the photometer in a specific measuring range at one or more specific wavelengths

Appendix: Photometer Test Protocols – BioPhotometer plus

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Eppendorf BioPhotometer® Plus

Photometrischer Test mit / Photometry test with Eppendorf Secondary-UV-VIS-Filter Bestell Nr. / Order No. 6131 931.008 Grenzen sind rückführbar auf NIST®: SRM 2034, SN 04-A, SRM 2031a, SN 667 und gemessen gegen Leerwert A0 / Limits are traceable to NIST: SRM 2034, SN 04-A, SRM 2031a, SN 667 and measured against Reference Blank A0.						
O. Leerwert / Reference		Testfilter	Filtercode	Wellenlänge / Wavelength		
		Blank AO		230 - 650 nm		
			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung Random / measurement deviation	
1. Wellenlänge / Wavelength accuracy						
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte $E^{\#}$ / Limiting values $A^{\#}$	Istwerte $E^{\#}$ / Actual values $A^{\#}$	Grenzwerte $VK^{\#}$ / Limiting values $CV^{\#}$	Istwerte $VK^{\#}$ / Actual values $CV^{\#}$
Probe 260 nm Sample 260 nm		260 ± 1 nm			≤ 3.0%	%
Probe 280 nm Sample 280 nm		280 ± 1 nm			≤ 3.0%	%
2. Photometrische Werte / Photometric values			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung / Random measurement deviation	
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte $E^{\#}$ / Limiting values $A^{\#}$	Istwerte $E^{\#}$ / Actual values $A^{\#}$	Grenzwerte $VK^{\#}$ / Limiting values $CV^{\#}$	Istwerte $VK^{\#}$ / Actual values $CV^{\#}$
Sample A 1		230 nm			≤ 3.0%	%
		260 nm				%
		280 nm				%
		340 nm				%
		405 nm				%
		490 nm				%
		550 nm				%
		595 nm				%
		650 nm				%
Sample A2		230 nm			≤ 1.0%	%
		260 nm				%
		280 nm				%
		340 nm				%
		405 nm				%
		490 nm				%
		550 nm				%
		595 nm				%
		650 nm				%
Sample A3		230 nm			≤ 1.5%	%
		260 nm				%
		280 nm				%
		340 nm				%
		405 nm				%
		490 nm				%
		550 nm				%
		595 nm				%
		650 nm				%

3. Elektrische Prüfung / Electric test			
		Grenzwerte / Limiting values	Istwerte / Actual values
Schutzimpedanz / Bonding impedance		≤ 0.2 Ohm	
Ableitstrom / Accessible current		≤ 3.5 mA	
Isolation		≥ 70 MOhm	
Datum / Date, Ort / Place	Geprüft durch / Tested by	Seriennummer / Serial No.	Software Version

Die Grenzwerte beinhalten die systematischen Fehler des Gerätes und der Mess- und Kalibriermittel. / Limits include the systematic error of the instrument and the measurement and calibration equipment.
Eppendorf ist zertifiziert nach DIN ISO 9001/ Eppendorf is certified according to DIN ISO 9001.

Appendix: Photometer Test Protocols – BioPhotometer D30

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Eppendorf BioPhotometer® D30

Photometrischer Test mit / Photometry test with Eppendorf Test-Filter (Satz Nr. / Set No.) Grenzen sind rückführbar auf NIST®: SRM 2034, SN 04-A, SN 667 und gemessen gegen Leerwert AO Limits are traceable to NIST: SRM 2034, SN 04-A, SN 667 and measured against Reference Blank AO						
O. Leerwert / Reference		Testfilter	Filtercode		Wellenlänge / Wavelength	
		Blank AO			260 - 800 nm	
			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung / Random measurement deviation	
1. Wellenlänge / Wavelength accuracy						
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E# / Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting values CV#	Istwerte VK# / Actual values CV#
Probe 260 nm Sample 260 nm		260 ± 1 nm			≤ 3.0%	%
Probe 280 nm Sample 280 nm		280 ± 1 nm			≤ 3.0%	%
2. Photometrische Werte / Photometric values			Systematische Messabweichung/ Systematic measurement deviation		Zufällige Messabweichung/ Random measurement deviation	
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E#*/ Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting values CV#	Istwerte VK# / Actual values CV#
Sample A1		260 nm			≤ 3.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		562 nm				%
		595 nm				%
Sample A2		260 nm			≤ 2.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		562 nm				%
		595 nm				%
Sample A3		260 nm			≤ 1.5%	%
		280 nm				%
		320 nm				%
		405 nm				%
		562 nm			≤ 3.0%	%
		595 nm				%

Ort / Location, Datum /Date	Geprüft durch / Tested by	Seriennummer / Serial No.	Software Version

Die Grenzwerte beinhalten die systematischen Fehler des Gerätes und der Mess- und Kalibriermittel. / Limits include the systematic error of the instrument and the measurement and calibration equipment.
Eppendorf ist zertifiziert nach DIN ISO 9001 / Eppendorf is certified according to DIN ISO 9001.

Appendix: Photometer Test Protocols – BioSpectrometer basic kinetic

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Eppendorf BioSpectrometer® basic/kinetic

Photometrischer Test mit I Photometry test with Eppendorf Test-Filter (Satz Nr. / Set No.)
Grenzen sind rückführbar auf NIST®: SRM 2034, SN 04-A, SN 667 und gemessen gegen Leerwert AO
Limits are traceable to NIST: SRM 2034, SN 04-A, SN 667 and measured against Reference Blank AO

O. Leerwert / Reference	Testfilter	Filtercode	Wellenlänge / Wavelength
	Blank AO		260 - 800 nm

			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung / Random measurement deviation	
1. Wellenlänge / Wavelength accuracy						
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E# / Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting Values CV#	Istwerte VK# / Actual values CV#
Probe 260 nm Sample 260 nm		260 ± 1 nm			≤ 3.0%	%
Probe 280 nm Sample 280 nm		280 ± 1 nm			≤ 3.0%	%
Probe 800 nm Sample 800 nm		800 ± 1 nm			≤ 3.0%	%
2. Photometrische Werte / Photometric values			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung / Random measurement deviation	
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E# / Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting values CV#	Istwerte VK# / Actual values CV#
Sample A1		260 nm			≤ 3.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm				%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%
Sample A2		260 nm			≤ 2.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm				%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%
Sample A3		260 nm			≤ 1.5%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm			≤ 3.0%	%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%

3. Thermische Prüfung mit / Thermal check with Testo® Temperature Set			
		Grenzwerte / Limiting values	Istwerte / Actual values
20 °C		19,7 °C – 20,3 °C	
25 °C		24,7 °C – 25,3 °C	
30 °C		29,7 °C – 30,3 °C	
37 °C		36,7 °C – 37,3 °C	
42 °C		41,7 °C – 42,3 °C	
Ort / Location, Datum / Date	Geprüft durch / Tested by	Seriennummer / Serial No.	Software Version

Die Grenzwerte beinhalten die systematischen Fehler des Gerätes und der Mess- und Kalibriermittel. / Limits include the systematic error of the instrument and the measurement and calibration equipment.
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Appendix: Photometer Test Protocols – BioSpectrometer fluorescence

Eppendorf BioSpectrometer® fluorescence

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Photometrischer Test mit / Photometry test with Eppendorf Test-Filter (Satz Nr. / Set No.) Grenzen sind rückführbar auf NIST®: SRM 2034, SN 04-A, SRM 2031a, SN 667 und gemessen gegen Leerwert AO Limits are traceable to NIST: SRM 2034, SN 04-A, SRM 2031a, SN 667 and measured against Reference Blank AO						
O. Leerwert / Reference		Testfilter	Filtercode		Wellenlänge / Wavelength	
		Blank AO			260 – 800 nm	
			Systematische Messabweichung / Systematic measurement deviation		Zufällige Messabweichung / Random measurement deviation	
1. Wellenlänge / Wavelength accuracy						
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E# / Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting values CV#	Istwerte VK# / Actual values CV#
Probe 260 nm Sample 260 nm		260 ± 1 nm			≤ 3.0%	%
Probe 280 nm Sample 280 nm		280 ± 1 nm			≤ 3.0%	%
Probe 800 nm Sample 800 nm		800 ± 1 nm			≤ 3.0%	%
2. Photometrische Werte / Photometric values			Systematische Messabweichung/ Systematic measurement deviation		Zufällige Messabweichung/ Random measurement deviation	
Testfilter	Filtercode	Wellenlänge/' Wavelength	Grenzwerte E#"/ Limiting values A#	Istwerte E#/ Actual values A#	Grenzwerte VK#/ Limiting values CV#	Istwerte VK#/ Actual values CV#
Sample A1		260 nm			≤ 3.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm				%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%
Sample A2		260 nm			≤ 2.0%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm				%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%
Sample A3		260 nm			≤ 1.5%	%
		280 nm				%
		320 nm				%
		405 nm				%
		550 nm			≤ 3.0%	%
		562 nm				%
		595 nm				%
		700 nm				%
		800 nm				%
3. Fluoreszenz Prüfung mit / Fluorescence check with						
Testfilter	Filtercode	Wellenlänge / Wavelength	Grenzwerte E# / Limiting values A#	Istwerte E# / Actual values A#	Grenzwerte VK# / Limiting values CV#	Istwerte VK# / Actual values CV#
Fluorescence		520 nm			≤ 3.0%	%
		560 nm				%
Ort / Location, Datum / Date		Geprüft durch / Test by		Seriennummer / Serial No.		Software Version

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Eppendorf ist zertifiziert nach DIN ISO 9001/ Eppendorf is certified according to DIN ISO 9001.

Ordering Information

Description	Order no. international	Order no. North America
Eppendorf BioPhotometer® plus,		
230 V / 50-60 Hz, mains/power plug Europe,	6132 000.008	
120 V / 50-60 Hz, mains/power plug North America		952000006
Eppendorf BioPhotometer® plus Reference filter set	6131 928.007	952010221
Eppendorf BioPhotometer® D30,		
230 V / 50-60 Hz, mains/power plug Europe,	6133 000.001	
120 V / 50-60 Hz, mains/power plug North America		6133000010
Eppendorf BioPhotometer® D30 Reference filter set	6133 928.004	6133928004
Eppendorf BioSpectrometer® basic,		
230 V / 50-60 Hz, mains/power plug Europe	6135 000.009	
120 V / 50-60 Hz, mains/power plug North America		6135000017
Eppendorf BioSpectrometer® kinetic,		
230 V / 50-60 Hz, mains/power plug Europe,	6136 000.002	
120 V / 50-60 Hz, mains/power plug North America		6136000010
Eppendorf BioSpectrometer® Reference filter set	6135 928.001	6135928001
Eppendorf BioSpectrometer® fluorescence,		
230 V / 50-60 Hz, mains/power plug Europe,	6137 000.006	
120 V / 50-60 Hz, mains/power plug North America		6137000014
Eppendorf BioSpectrometer® fluorescence Reference filter set	6137 928.009	6137928009
Thermo Printer DPU-S445 incl.		
mains /power plug and printer cable 230 V, EU	6135 011.000	
115 V / 100 V, mains/power plug North America		6135010004
Thermo paper 5 rolls	0013 021.566	952010409

Your local distributor: www.eppendorf.com/contact
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