Userguide

No 13 | BioPhotometer plus

Troubleshooting Guide for the measurement of nucleic acids with the BioPhotometer plus

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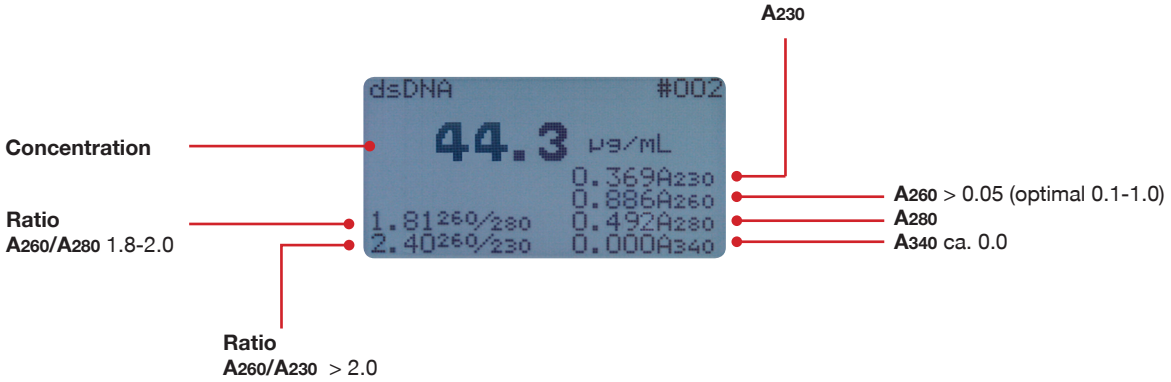
Abstract

This Troubleshooting Guide will help you achieve reliable results using the Eppendorf BioPhotometer plus. It explains in detail the basic functions of the instrument. Additionally, the critical factors for attaining a precise measurement are summarized and recommendations are given on how to solve problems that might be encountered.

Important information that you can derive from the display

After you have carried out your measurement, you will find the following data in the display:



eppendorf

A260 nm

Maximum absorption of nucleic acids

The optimal measuring range for precise measurements lies between 0.1 and 1.0.

The dependence of the light absorption on the concentration of the absorbing substance in diluted solutions is described by Lambert-Beers law: $A = \varepsilon \cdot c \cdot d$

The DNA concentration in a 50 µL sample must be at least 2.5 µg/mL (= 125 ng) of dsDNA (A_{260} = 1 corresponds to 50 µg/mL dsDNA).

→ Is the value between 0.1 - 1.0? → If not, either dilute or concentrate your sample until it lies within this range. At high concentrations you can also turn the UVette® using the 2 mm path length.

With extinctions of less than 0.05, all sources of error, regardless of the device (e.g. imprecisions by pipetting, particulate matter ect.), become too significant to receive exact measurements.

Lambert-Beers law $A = \log \frac{I_0}{I} = \varepsilon \cdot c \cdot d$

A measured extinction (Absorption)
I_0 intensity of the entering luminous flux;
I intensity of the exiting light after passing through the sample;
c molar concentration of the absorbing substance [mol/L]
d layer thickness [cm]
ε molar extinction coefficient [L mol $^{-1}$ cm $^{-1}$]

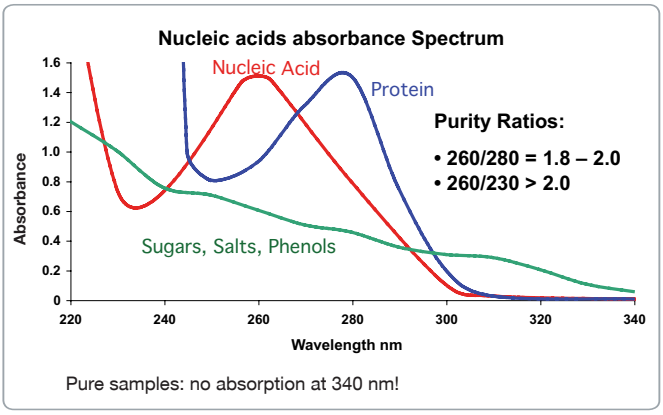
A280 nm

Maximum absorption of proteins and phenol

Reference measuring value for determination of the purity of the nucleic acid sample:

Ratio A_{260}/A_{280} → Is the value for DNA around 1.8 and around 2.0 for RNA? → If this value is lower, this may indicate contamination by proteins or phenol. Purify your sample.

The ratio for pure DNA is 1.8 and 2.0 for RNA. Lower values indicate contamination by proteins (aromatic groups) and phenol. A value of 1.5 corresponds to a 50% protein/DNA solution.



Pure samples: no absorption at 340 nm!

A230 nm

Maximum absorption of carbohydrates

Reference measuring value for determination of the purity of the nucleic acid sample:

Ratio A_{260}/A_{230} → Is the value > 2.0? → If this value is less than 2.0, your sample could be contaminated by carbohydrates, salts or organic solvents. Purify your sample.

The ratio should be more than 2.0 for pure DNA and RNA. Values less than 2.0 indicate contamination by sugar, salt or organic solvents.

A340 nm

Reading, measured value for turbidity in the measuring solution sample.

→ Is the value close to 0.0? → If this value is not close to 0.0, you have particulate matter in your sample. Purify the sample. Alternatively, you can activate the „A_{340} correction“ in the parameters. An arrow symbol then appears next to the E_{340} display.

The value should be close to 0.0. Anything else is an indication of particulate matter. One can set a turbidity correction with the parameter setting „Corr. with A_{340}“.

Error messages in the results display

● +++++	The measured absorption is greater than 3.0.	● Dilute your sample. ● Check whether your cuvette has a light beam height of 8.5 mm. ● Clean the cuvette shaft. ● Check whether the cuvette has been inserted in the correct direction (Measuring window in the direction of the light path).
● !!!!!	Calculated value cannot be displayed (Value too high).	Check your set parameters. The factor set may be too high.
● – – – – –	Ratio cannot be calculated because the absorption of one of the values used for the calculation is 0.0 or greater than 3.0.	Repeat the measurement with an appropriately diluted sample.

Most frequent sources of errors

The dilution

● We recommend a dilution factor of 1:10 to 1:50, as the pipetting error is too great with higher dilution ratios.

● Was the factor entered correctly into the device?

● Have the pipettes been calibrated?

Very important!
Mixing the sample

● The nucleic acid sample should be briefly vortexed prior to dilution in order to avoid possible concentration gradient in the sample.

● Before transferring the sample into the cuvette vortex briefly again prior to measurement to prevent concentration fluctuations caused by long storage of the sample.

Tips & Tricks

● „Sources of errors with photometric measurement of nucleic acids“
BioNews Application Note 17:1

● www.biophotometer.info
www.eppendorf.com
(Applications)

The measuring medium

● The absorption behavior of nucleic acids is influenced by the pH value and the ionic strength of the buffer. One can thus only obtain precise concentrations under controlled pH conditions and with solutions with low ionic strengths, e.g. with 10 mM Tris-HCl pH 8.0. Because water is not pH-stable, fluctuating measurement results may occur.

● Some buffers may exhibit self-absorption in the UV range. In order to avoid inaccuracies, use the same buffer in which the sample was resuspended/ eluted following isolation for the blank and the sample.

The cuvettes and what one should be aware of

● The light beam height of the cuvette must match with that of the BioPhotometer plus (8.5 mm).

● The correct layer thickness of the cuvette must be set on the BioPhotometer plus (Parameters). When changing from 10 mm to 2 mm and vice versa, a new blank must be measured.

● Quartz glass cuvettes should be carefully cleaned, so that they are free of DNA/RNA contamination.

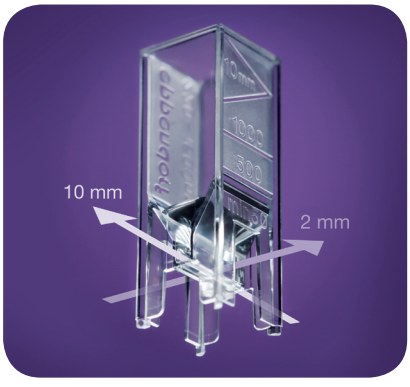
● Plastic cuvettes such as the Eppendorf UVette may not be reused too often. We recommend using the UVette a maximum of five times. UV light causes a change of the optical properties of the plastic. This can lead to measurement errors.

● The light path of the cuvette must be oriented in the direction of the light beam of the BioPhotometer plus.

● The blank and the sample should be measured in the same cuvette in order to avoid fluctuations of the measured values between cuvettes.

● The specific sample volume for the cuvette must be observed. For the UVette this is 50 µl, as stray light effects may otherwise occur.

● Air bubbles or other visible particles in the cuvette must be avoided.



Other causes

- Is the BioPhotometer plus lying flat?
- Is the cuvette shaft clean, so that the cuvette fits correctly in the shaft?
- Have the pipettes been correctly calibrated?
- Is the pipette piston clean?

Still in doubt?

Reference measurement

Carry out a reference measurement in comparison to a standard nucleic sample with a defined concentration or use the UV-VIS filter set offered by Eppendorf.

Standard nucleic acid sample

This is how it‘s done:

- Buffer: 10 mM Tris-HCl pH 8.0 or TE buffer pH 8.0.
- Dilution: 2 + 48 µL (>125 ng DNA per 50 µL sample).
- Same cuvette for blank and sample.
- Carry out sample dilution in Eppendorf Tube.
- Vortex sample well.
- Avoid air bubbles in the cuvette.

UV-VIS Filter Test

When handling errors have been eliminated, you can determine technical errors of the BioPhotometer plus with reference measurements in comparison with reference materials. For this purpose Eppendorf offers the Secondary UV-VIS FIter Set. The measuring values thus determined are compared with the limiting values established for each filter. This makes it possible to obtain information concerning the accuracy and precision of the device. You will find more information in the operating manual and tips for carrying out such a test in our Biophotometer plus userguide.

Still need assistance?

Please contact your local Eppendorf organization or the Eppendorf Application Support at support@eppendorf.com.

Biophotometer plus AU010 userguide

„Evaluating the functionality of the Eppendorf BioPhotometer plus using the Secondary UV-VIS Filter Set“

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