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Eppendorf µCuvette™ G1.0 -

A new micro-volume system for highly precise photometric determination of nucleic acids or proteins in the Eppendorf BioPhotometer® and Eppendorf BioSpectrometer®.

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Abstract

The use of the microliter measurement cell µCuvette, in combination with the BioPhotometer or BioSpectrometer, enables determination of high concentrations of nucleic acids or proteins using minimum volumes. In order to achieve optimum results, the measurement principle will be elaborated in this application note, and use and handling of the µCuvette are described in detail. Possible factors which may influence measurements, as well as individual instrument parameters, will also be discussed. In addition, comparative measurements with other cuvettes and microvolume cuvettes were performed, with a focus on self absorption, precision and accuracy.

Introduction

Following isolation of nucleic acids or proteins, it is usually necessary to determine the concentration of these biomolecules photometrically in order to prepare them for downstream applications. Since the samples are often highly concentrated following isolation, dilution is frequently required.

In order to avoid faulty calculation of the originating concentration, and thus faulty calculation for subsequent applications, the individual dilution steps need to be executed with extreme accuracy. The challenge lies in the fact that most samples become unusable following dilution; renewed concentration of the samples would prove rather labor-intensive. In order to avoid such serial dilutions, microliter measurement systems may be used which are capable of measuring highly concentrated samples without the need for dilution. For these measurements, a few microliters suffice. This is made possible by the shortened light path employed by the microliter measurement systems. As shown in figure 1, when a certain sample concentration is reached in a normal cuvette featuring a 10 mm light path, light is unable to pass through the solution and therefore cannot reach the detector within the photometer. In the case of a microliter measurement cell with abbreviated path length, the path is not long enough to block the light from passing through the very same sample. Therefore, the light reaches the detector, and the concentration can be determined under consideration of the abbreviated light path.

Part 1: Handling of the µCuvette

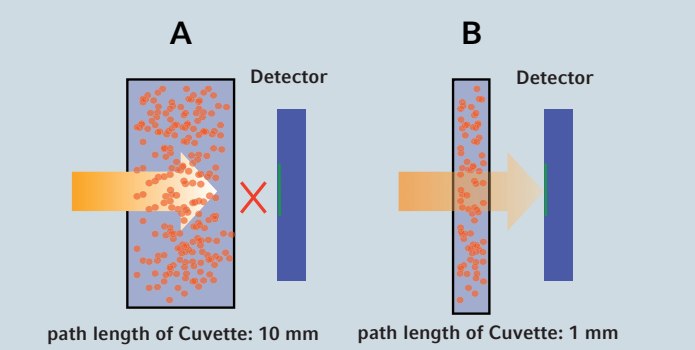

Figure 1: Abbreviation of the light path for photometric determination of highly concentrated samples (not to scale):
A) Standard cuvette
B) Microliter measurement cell
While the sample concentration may not be determined in a cuvette with a path length of 10 mm, the sample can be measured in a cuvette with a path length of 1 mm.

In order to implement the measurement principle depicted in figure 1, Eppendorf developed the µCuvette (figure 2). The µCuvette consists of two arms; at the end of each resides a small quartz plate. To facilitate sample application onto the sample application area, two black markings can be found at the end of both arms.

Measurement range of the µCuvette

The 1 mm light path of the µCuvette enables the measurement of 10-fold higher sample concentrations than would be possible with standard cuvettes with their conventional 10 mm path length.

path, or a µCuvette with 1 mm light path, respectively. Further, the optimum concentration range which would span a respective linear standard curve is shown. The µCuvette was designed exclusively for use in the BioPhotometer and BioPhotometer plus, as well as in the BioSpectrometer,

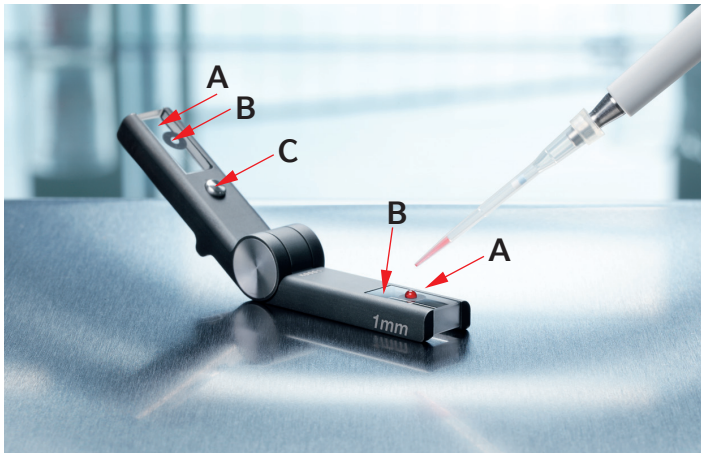

Figure 2: The µCuvette:
A: Sample application area and optical measurement window
B: Quartz plates
C: Spacer

since the light beam of these instruments is optimized to the measurement window of the µCuvette. Due to optimization of the light beam in BioSpectrometers, even smaller volumes may be used for measurement than when using BioPhotometers.

Table1: Measurement range for selected biomolecules in the Eppendorf BioSpectrometer

Sample	µCuvette – 1 mm path length (0.005-3E)	µCuvette – 1 mm path length – optimum (0.05-2E)	Standardküvette z.B. Eppendorf UVette - 10 mm path length (0.005-3E)	Standardküvette z.B. Eppendorf UVette - 10 mm path length – optimum (0.05-2E)
dsDNA	2.5 – 1500 µg/mL	25-1000 µg/mL	0.25 – 150 µg/mL	2.5-100 µg/mL
RNA	2 – 1200 µg/mL	20-800 µg/mL	0.2 – 120 µg/ml	2-80 µg/mL
Protein (BSA)	0.075 - 45 mg/mL	0.75-30 mg/mL	0.0075- 4,5 mg/mL	0.075-3 mg/mL

This also means that one and the same sample will yield a 10-fold lower absorbance when measured in a 1 mm light path, compared to a 10 mm light path. Table 1 lists the ranges theoretically possible for measurement of selected biomolecules. These values refer to the instrument specifications of the BioPhotometer and BioSpectrometer, in combination with cuvettes featuring a 10 mm light

Apart from avoiding cumbersome serial dilutions, the µCuvette has a further advantage: only small volumes are required for measurement. Table 2 outlines sample volumes required for measurement using the BioPhotometers or the BioSpectrometers, respectively. Following the measurement process, samples may be easily recovered with a pipette for further use.

Table 2: Sample volumes required for determination in Eppendorf Photometers and Spectrometers (max. 10 µL)

Sample	Eppendorf BioPhotometer	Eppendorf BioSpectrometer
Nucleic acids	≥ 2 µL	≥ 1.5 µL
Protein	≥ 4 µL	≥ 3 µL

The specifications regarding sample volumes refer to aqueous solutions. In the case of DNA solutions containing a relatively high protein contamination, the volume may be slightly higher. No detergent-containing solutions should be used. Also, highly concentrated nucleic acid solutions could display reduced surface tension; therefore, the volume may need to be increased in order to guarantee the formation of the necessary liquid column.

Measurements performed in the BioPhotometer or BioSpectrometer

When using the µCuvette in the BioSpectrometer or BioPhotometer, the respective instrument parameters should be set in advance. It is of special importance to set the optical path length to 1 mm; the absorbances measured may then be directly converted to sample concentration by taking the shorter path length into account. If background signals are expected, for example, due to contaminations, it is recommended to activate the background correction function in both instruments. With low concentrations, even a small background signal may significantly influence the result. However, since the actual sample concentration is generally unknown prior to measurement, background correction is recommended in all cases.

The µCuvette in the BioPhotometer plus and the BioPhotometer 6131

Figure 3 shows the required parameter settings on the BioPhotometer plus for the method of double-stranded DNA (dsDNA) measurement.

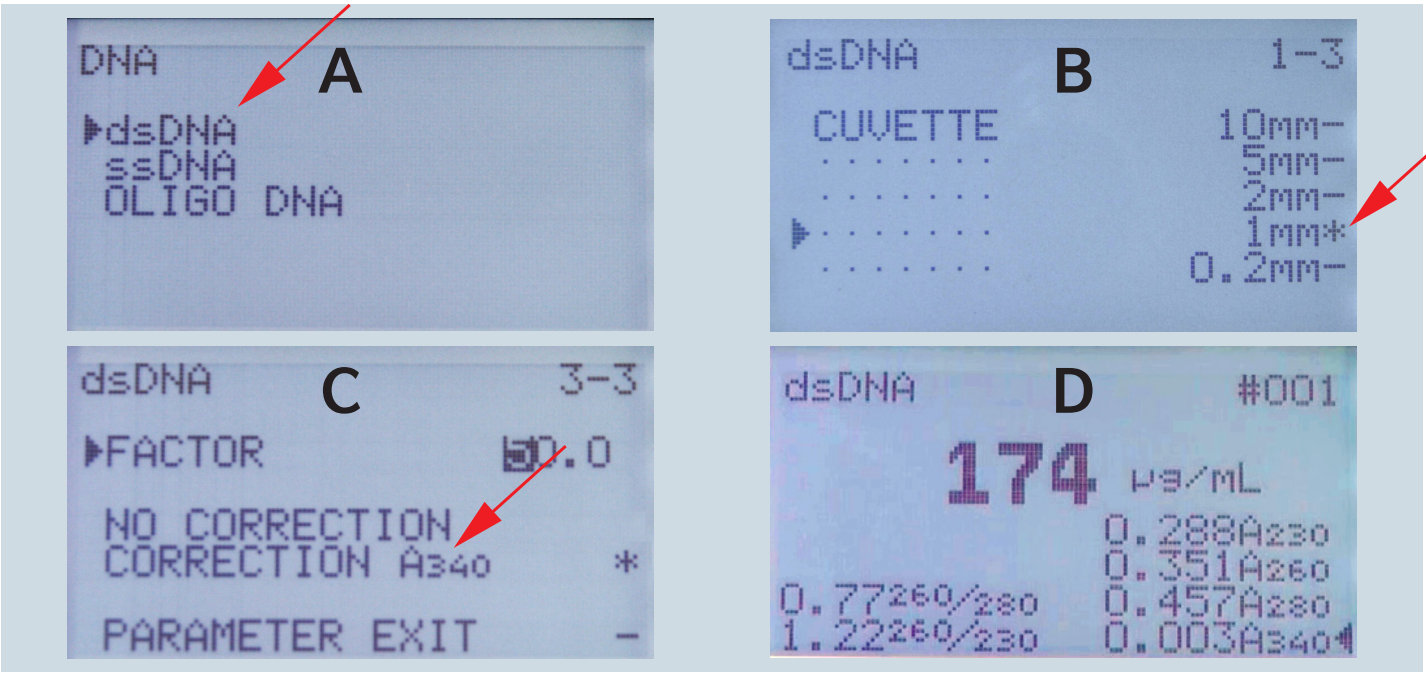

Fig. 3: Setting of parameters on the BioPhotometer plus for use of the 1 mm light path and for activation of background correction:

A) Select method group DNA and then move the cursor keys to the method "dsDNA". Press the key "Parameter".

B) Move the cursor keys to "1mm" and select this light path by pressing "enter" (display 1-3).

C) Take the cursor keys to parameter display 3-3 and select background correction "Corrections A340" by pressing "enter". Leave the parameter area by pressing the parameter-key again.

D) Example of a dsDNA concentration determination with 1 mm light path and activated background correction (black arrow: measurement at 340 nm). In addition to absorbance measurement at 260 nm, the absorbances at 230 nm and 280 nm are also determined to calculate the 260/230 and 260/280 ratios, respectively.

The precursor model BioPhotometer features analog parameter selection. However, it may happen that in combination with older software, direct selection of the 1 mm light path is not possible. In this case, a virtual dilution is required. Prior to measurement, please program a 1:10 dilution
(e.g. 1 µl sample + 9 µl diluent). This way, you will obtain the same result as with a directly programmable light path of 1 mm.

Measurement in the BioSpectrometer
In the BioSpectrometer, the parameters are defined directly prior to the actual measurement. In contrast to the BioPhotometer plus or the BioPhotometer the full absorbance spectrum of the sample may be displayed for a defined area, and the wavelength for background correction may be selected freely (figure 4). For ease of use, the BioSpectrometer features a pre-programmed method for the measurement of dsDNA using the 1 mm path length.

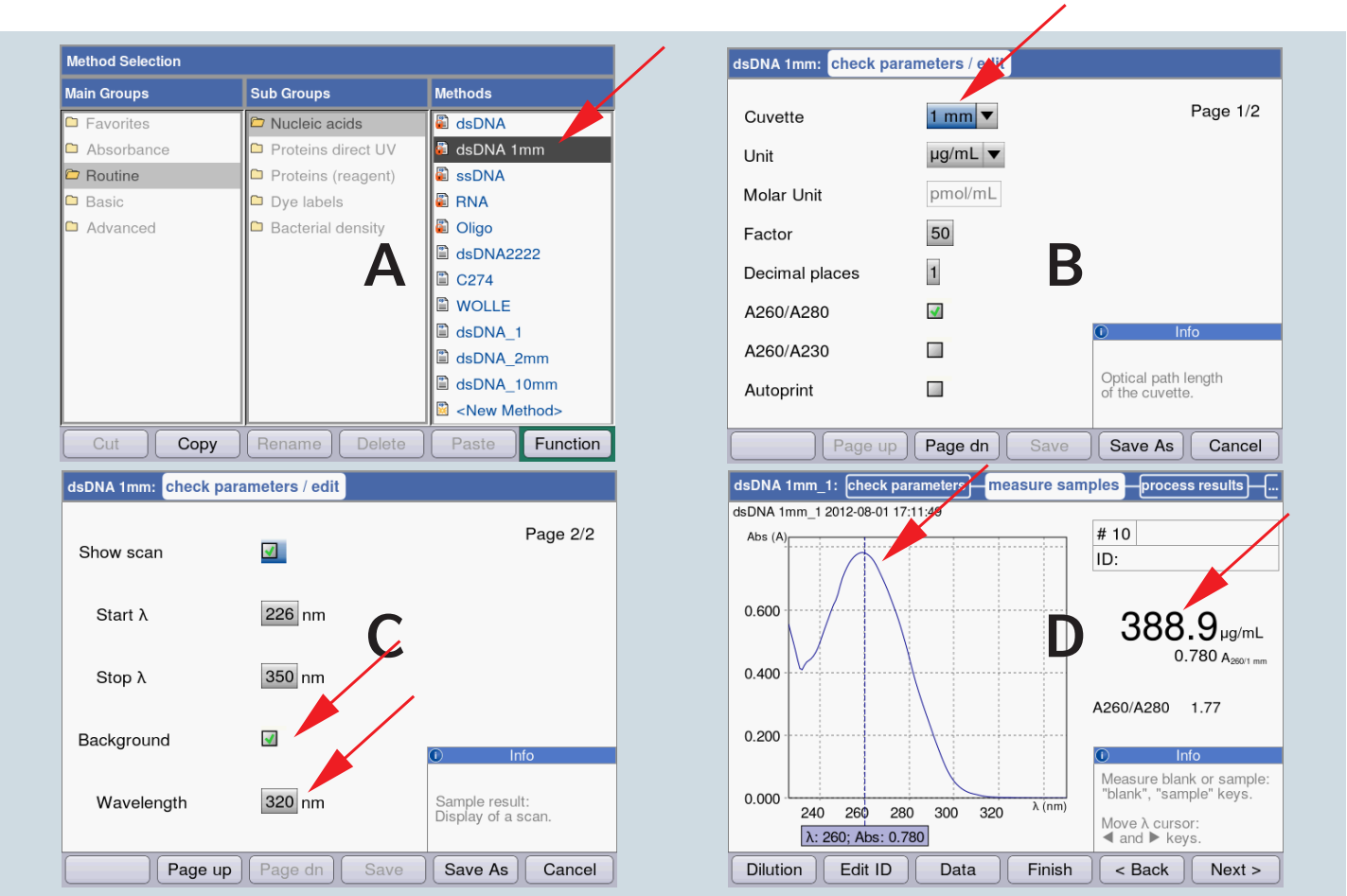

Fig. 4: Parameter selection on the BioSpectrometer for application of the 1 mm light path and for activation of background correction:
A) Selection of method dsDNA_1mm
B) Select optical path length to 1 mm (only required if dsDNA_1mm is not employed)
C) If desired, you may activate the scan display and/or background correction
D) Example of a measurement of a dsDNA-solution using the µCuvette in the BioSpectrometer

Measurement process in the BioPhotometer/BioSpectrometer

The blank solution is applied to the appropriate black-rimmed are of the µCuvette. Then both arms are folded together, ensuring that a liquid column will form between the two sample carriers, as shown in the diagram in figure 5.

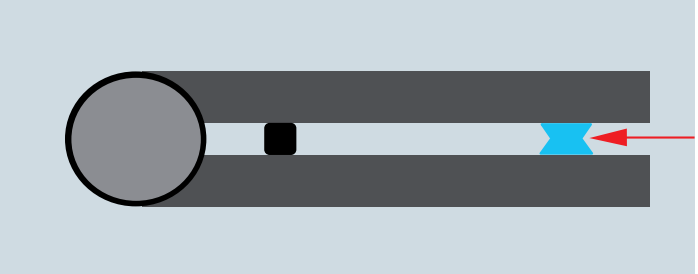

Fig. 5: Diagram of the µCuvette.
The sample has formed a liquid column (red arrow). The height of the column is equivalent to a light path of 1 mm.

Prior to measurement, the sample should be for air bubbles and/or particles. If the sample is free of such contaminants, the µCuvette may be transferred to the cuvette shaft in order to measure the blank. Subsequently, the blank solution is removed with a lint-free tissue. Now the sample may be applied and measured (figure 6).

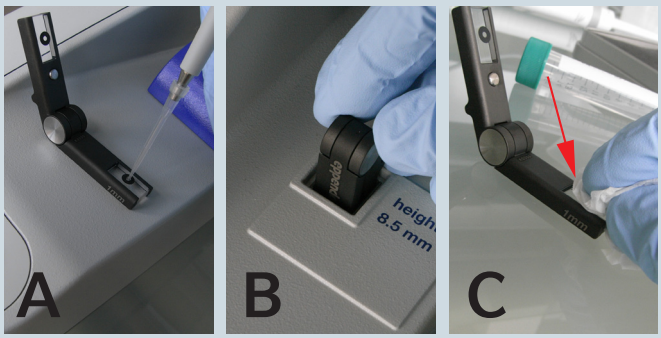

Figure 6: Measurement process with the µCuvette:
A) Sample application
B) Insertion into the cuvette shaft
C) Removal of the blank or sample solution

Problems with non-formation of the liquid column

In the case where no sample column is formed, no proper result can be obtained. Figure 7A shows such a result.

If the liquid column has formed properly, a result such as the one shown in figure 7B may be expected. In both cases the identical dsDNA sample was measured.

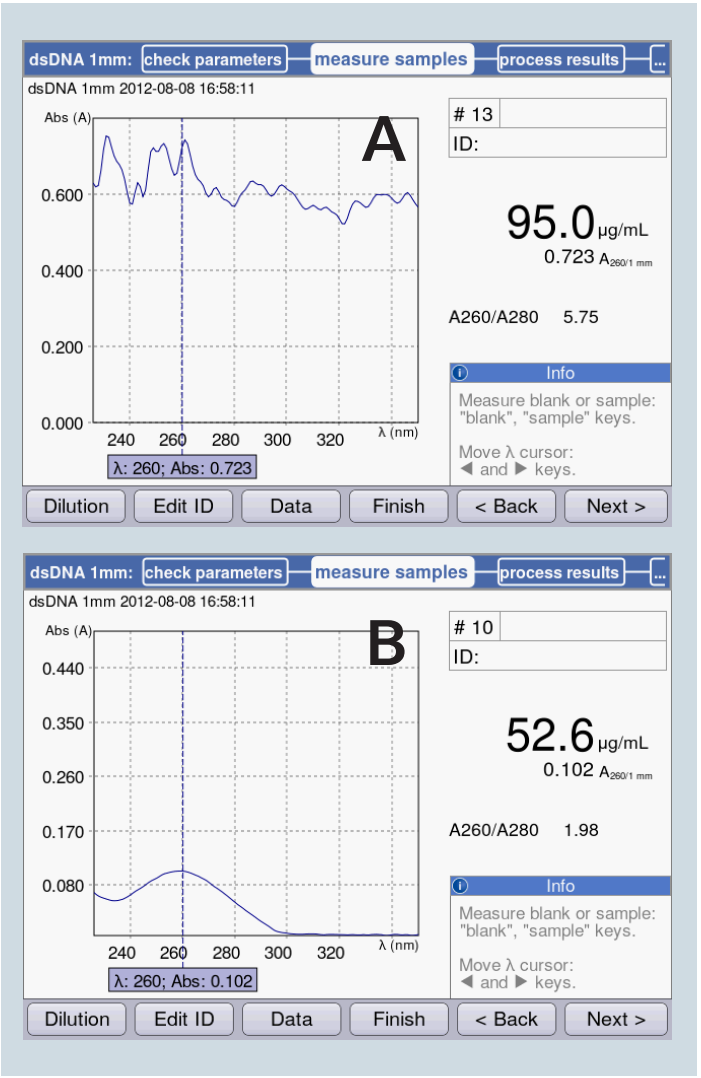

Fig. 7: Measurement results with dsDNA samples:
A) Without formation of liquid column
B) With formation of liquid column

The result shown in figure 7 represents the typical absorbance spectrum of a dsDNA-solution. The sample concentration, as well as the 260/280 ratio could be determined easily. Neither was possible in the measurement depicted in figure 7A, since the liquid column had not formed.

Handling and cleaning
Gloves are recommended during the measurement process in order to prevent contaminations in the sample application area. Similar to measurements in standard cuvettes with a 100 light path, frozen samples should always be completely thawed and subsequently mixed sufficiently. Ideally, sample and µCuvette are at the same temperature when measurement is initiated.
Between two measurements, the sample carriers need to be cleaned, and remaining liquid needs to be removed entirely, using lint-free wipes. At the same time it is recommended to ensure that the light path is free of lint or smears. Contaminations are best prevented by cleaning the sample carriers of the µCuvette with deionized water following each measurement. However, if contaminations, for example from nucleic acids, are found repeatedly, the µCuvette may also be cleaned with a wipe containing 6 % sodium hypochlorite, which is able to remove especially persistent contaminations. However, since no edges are present in the area of the sample carrier, the accumulation of residue is extremely unlikely.
Further information on cleaning may also be found in the user manual of the µCuvette.

Part 2: Experiments featuring the µCuvette

Autoabsorbance of the µCuvette in comparison with cuvettes made by competing manufacturers
In order to determine the dynamic range of the µCuvette, autoabsorbance was measured across the range between 200 and 400 nm in the BioSpectrometer. The absorbance spectrum thus obtained was compared to those of other cuvettes, or microcuvettes, respectively (figure 8).
Each cuvette contained water and was measured against air (empty cuvette shaft).

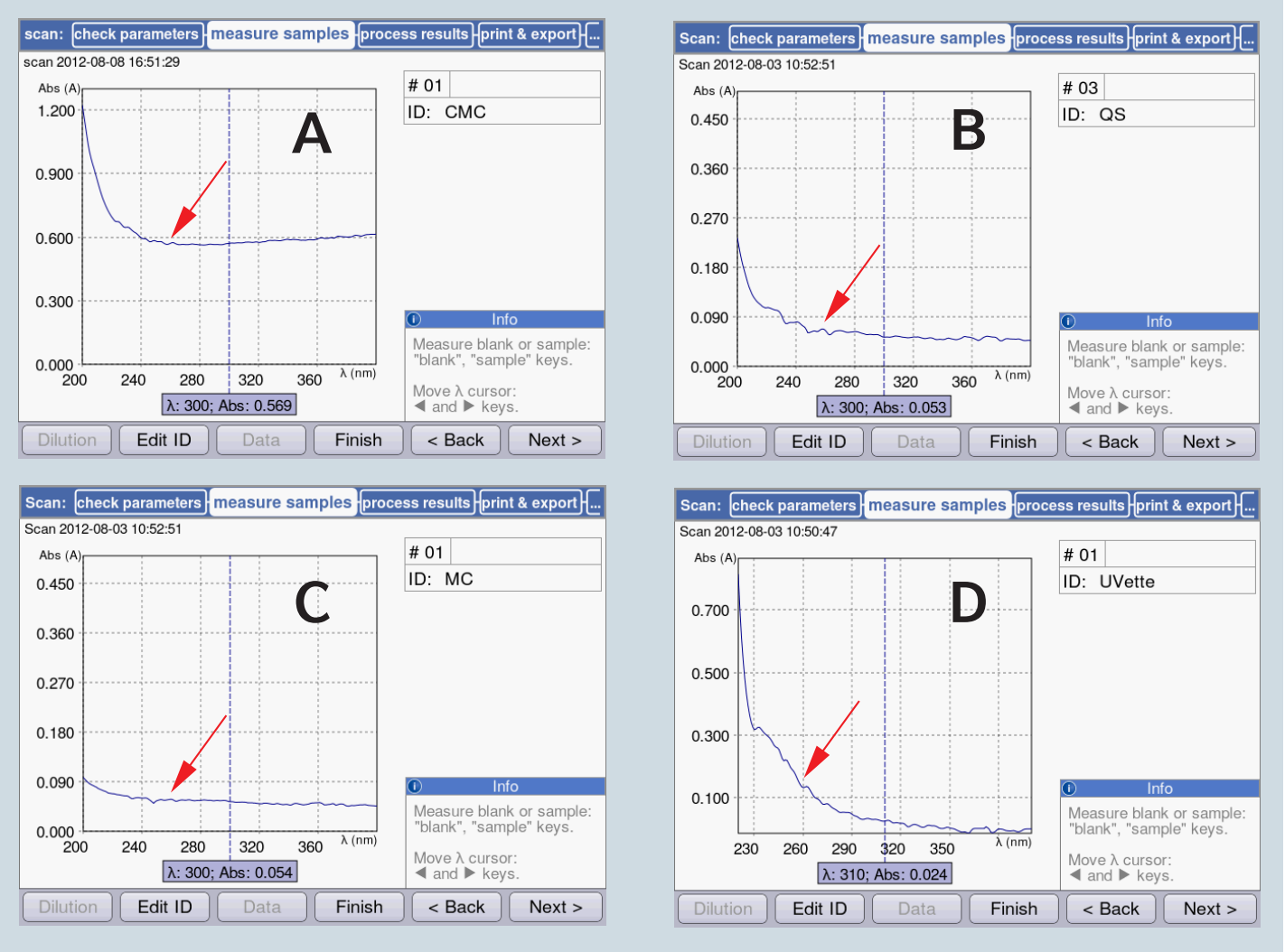

Figure 8: Comparison of autoabsorbance of the µCuvette with that of other cuvettes. The red arrow indicates the autoabsorbance of the cuvette at 260 nm:

A) Microcuvette by a competing manufacturer

B) Quartz cuvette

C) µCuvette

D) UVette

As shown in figure 8, the autoabsorbance of the µCuvette is comparable to that of of a quartz cuvette (<0.1 A at 260 nm), and it is significantly lower than the autoabsorbance of the microcuvette made by a different manufacturer (approx. 0.6 A at 260 nm).

Furthermore, the UVette shows very low autoabsorbance for a plastic cuvette, with only 0.12 A.
Owing to the low autoabsorbance of the µCuvette, practically the entire spectrum of the BioPhotometer, as well as the BioSpectrometer, may be utilized.

Deviations of the µCuvette in comparison with a microliter measurement cell by a different manufacturer by means of absorbance determinations at 260 nm.
For comparative measurements of an ATP stock solution, different absorbance values of 0.1, 0.5, 1.0, 1.5 and 2.0, with reference to the 1 mm light path, were selected. To this end, the ATP solution was diluted accordingly in 0.1 M Tris buffer, pH 7.27. This buffer was also used as the blank. Furthermore, all solutions were also diluted 1:10 and measured in a quartz-ultra-micro cuvette with a 10 mm light path.

These values subsequently served as reference values for the measurements performed in the microliter measurement cells. All measurements were performed with activated background correction in the BioPhotometer as well as in the BioSpectrometer, using the pre-programmed method dsDNA in both instruments. For measurements in the microliter measurement cells, 3 µL were employed and 100 µL were used in the ultra-micro cuvette. Measurements were repeated 10 times for each concentration and each cuvette, respectively. The results are shown in tables 3 a-e.

Table 3a: Measurements at 260 nm: E = 0.1

	Ultra-micro cuvette (UM)		Microliter measurement cell 1 mm light path		µCuvette	
Instrument	BioPhotometer	BioSpectrometer	Biophotometer	BioSpectrometer	BioPhotometer	BioSpectrometer
Average (from 10 measurements)	0.104	0.098	0.108	0.107	0.105	0.102
Standard deviation	0.002	0.001	0.002	0.004	0.002	0.001
[%] CV	1.810	1.521	1.824	3.443	1.458	0.849
Deviation from Ultra micro cuvette			0.004	0.009	0.001	0.004
Deviation from Ultra micro cuvette in [%]			3.85	9.18	0.96	4.08

Table 3b: Measurements at 260 nm: E = 0.5

	Ultra-micro cuvette (UM)		Microliter measurement cell 1 mm light path		µCuvette	
Instrument	BioPhotometer	BioSpectrometer	Biophotometer	BioSpectrometer	BioPhotometer	BioSpectrometer
Average (from 10 measurements)	0.505	0.506	0.514	0.510	0.509	0.507
Standard deviation	0.001	0.001	0.006	0.007	0.001	0.001
[%] CV	0.288	0.287	1.109	1.465	0.268	0.242
Deviation from Ultra micro cuvette			0.009	0.004	0.004	0.001
Deviation from Ultra micro cuvette in [%]			1.78	0.79	0.79	0.19

Table 3c: Measurements at 260 nm: E = 1.0

	Ultra-micro cuvette (UM)		Microliter measurement cell 1 mm light path		µCuvette	
Instrument	BioPhotometer	BioSpectrometer	Biophotometer	BioSpectrometer	BioPhotometer	BioSpectrometer
Average (from 10 measurements)	1.010	1.009	1.018	1.015	1.009	1.007
Standard deviation	0.006	0.006	0.008	0.007	0.003	0.002
[%] CV	0.581	0.561	0.791	0.739	0.252	0.212
Deviation from Ultra micro cuvette			0.008	0.006	0.001	0.002
Deviation from Ultra micro cuvette in [%]			0.79	0.59	0.09	0.19

Table 3d: Measurements at 260 nm: E = 1.5

	Ultra-micro cuvette (UM)		Microliter measurement cell 1 mm light path		µCuvette	
Instrument	BioPhotometer	BioSpectrometer	Biophotometer	BioSpectrometer	BioPhotometer	BioSpectrometer
Average (from 10 measurements)	1.509	1.502	1.500	1.481	1.511	1.498
Standard deviation	0.008	0.007	0.018	0.005	0.005	0.003
[%] CV	0.552	0.462	1.187	0.337	0.312	0.200
Deviation from Ultra micro cuvette			0.009	0.021	0.002	0.004
Deviation from Ultra micro cuvette in [%]			0.60	1.39	0.13	0.27

Table 3e: Measurements at 260 nm: E = 2.0

	Ultra-micro cuvette (UM)		Microliter measurement cell 1 mm light path		µCuvette	
Instrument	BioPhotometer	BioSpectrometer	Biophotometer	BioSpectrometer	BioPhotometer	BioSpectrometer
Average (from 10 measurements)	2.004	1.974	1.936	1.910	1.988	1.952
Standard deviation	0.004	0.012	0.043	0.008	0.005	0.006
[%] CV	0.209	0.606	2.213	0.418	0.270	0.307
Deviation from Ultra micro cuvette			0.068	0.064	0.016	0.022
Deviation from Ultra micro cuvette in [%]			3.39	3.24	0.79	1.11

With regards to the results of the measurements, the µCuvette shows high agreement with the expected results, and high agreement with the results obtained using the ultra-micro cuvette. Thus, the deviations of the µCuvette were lower than those of the microliter measurement cell by the competing manufacturer.
The low standard deviations underline the high level of precision possible when using the µCuvette. Further, the values for the µCuvette were comparable to those obtained with the ultra-micro cuvette made from quartz. The standard deviations achieved with the µCuvette were also lower than those obtained with the microliter measurement cell by the competing manufacturer.

Conclusion

The µCuvette represents a microvolume cuvette which is optimally suited for photometric measurements of small volumes of highly concentrated samples. Therefore, and owing to its easy handling, the µCuvette and the Eppendorf Photometers complement each other to form an optimal microvolume measurement system. This is especially true for the determination of nucleic acids and proteins. Due to the low autoabsorbance of the µCuvette, a dynamic range is achieved which is generally only possible with quartz cuvettes.
Furthermore, the µCuvette performs at a high level of accuracy and precision.

Ordering information

Description	International Order no.	North America Order no.
Eppendorf µCuvette™ G1.0		
Eppendorf microvolume measuring cell for Eppendorf BioPhotometer and BioSpectrometer	6138 000.018	6138000018
Eppendorf BioSpectrometer® basic		
230 V/50-60 Hz, mains plug for Europe, additional mains/power connections available	6135 000.009	-
120 V/50–60 Hz, mains plug for North America	6135 000.017	6135000017
Eppendorf BioSpectrometer® kinetic		
230 V/50-60 Hz, mains plug for Europe, additional mains/power connections available	6136 000.002	-
120 V/50–60 Hz, mains plug for North America	6136 000.010	6136000010
Eppendorf BioPhotometer® plus		
230 V / 50-60 Hz, mains plug for Europe, additional mains/power connections available	6132 000.008	-
120 V/50–60 Hz, mains plug for North America	6132 000.016	952000006
Eppendorf UVette® 220 nm–1,600 nm		
Original Eppendorf disposable cuvette, individually packaged, certified PCR clean and protein-free, 80 pcs.	0030 106.300	952010051
UVette® routine pack 220 nm–1,600 nm		
Eppendorf Quality purity grade, disposable box, 200 pcs.	0030 106.318	952010069
Eppendorf µCuvette™ G1.0 & Eppendorf BioPhotometer® plus		
Eppendorf microvolume measuring cells and BioPhotometer plus,		
230 V/50–60 Hz, mains plug for Europe, additional mains/power connections available	6132 000.961	-
120 V/50–60 Hz, mains plug for North America	6132 000.962	6132000996
Eppendorf µCuvette™ G1.0 & Eppendorf BioSpectrometer® basic		
Eppendorf microvolume measuring cells and BioSpectrometer basic,		
230 V/50–60 Hz, mains plug for Europe, additional mains/power connections available	6135 000.904	-
120 V/50–60 Hz, mains plug for North America	6135 000.905	6132000923