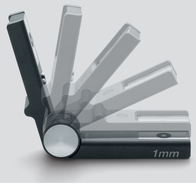
Correct Usage of Eppendorf µCuvette® G1.0



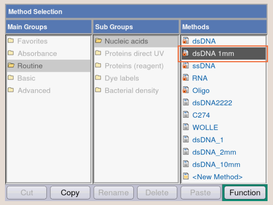
Eppendorf µCuvette G1.0 is a micro volume system that is designed for precise photometric measurement of nucleic acids and proteins. High concentration dsDNA sample of ≥ 1.5 µl can be measured without a need for sample dilution. Eppendorf µCuvette G1.0 is optimized to be used on Eppendorf BioPhotometer®, Eppendorf BioPhotometer plus, Eppendorf BioPhotometer D30, and Eppendorf BioSpectrometer® basic, kinetic, and fluorescence.

Cleaning and Maintenance of Eppendorf µCuvette G1.0

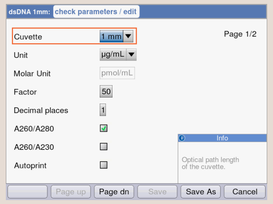
- Clean the sample loading area with deionized water and Kimwipes prior to and after sample measurement.
- Avoid touching the quartz surfaces.
- Do not autoclave or use ultrasonic bath for cleaning purpose.
- To remove stubborn stains, use 6 % sodium hypochlorite or 70 % ethanol.
- µCuvette is not resistant to acetone, strong acids, strong bases, peroxide solutions, and peroxide gases.
- Do not soak the µCuvette into cleaning agents.

Operation Procedure of Eppendorf µCuvette G1.0

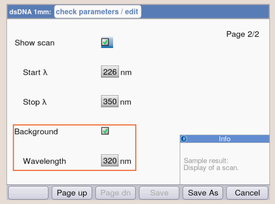
A) Parameters setting on Eppendorf BioSpectrometer



1. Selection of measurement method: “dsDNA 1 mm”



2. Selection of cuvette light path length: “1 mm”

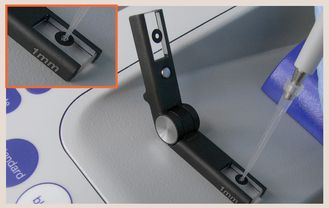


3. Activation of background correction at 320 nm

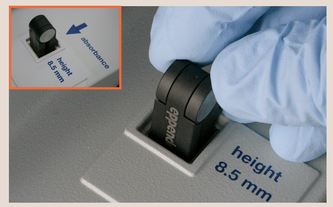
B) Sample volume required for measurement

Sample	Eppendorf BioSpectrometer and Eppendorf BioPhotometer D30	Eppendorf BioPhotometer and Eppendorf BioPhotometer plus
Nucleic acids	≥ 1.5 µL	≥ 2.0 µL
Proteins	≥ 3.0 µL	≥ 4.0 µL

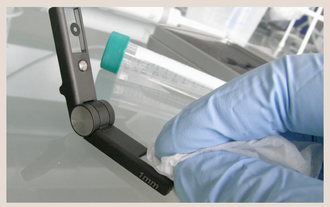
C) Measurement procedure on Eppendorf BioSpectrometer



1. Open both arms and place the µCuvette onto a flat surface.
2. Ensure that the sample loading area is free from fingerprints, dust, and scratches.
3. Load the blank, standard, or sample onto the spot in the middle of the black hydrophobic ring.
4. Make certain that there is no bubble in the sample and then close both arms of the µCuvette. A “click” sound ensures that the µCuvette is closed securely.
5. Make sure a liquid column is formed in between both arms of the µCuvette.



6. Next, insert the µCuvette all the way down to the bottom of the cuvette shaft.
7. Make certain that the µCuvette is oriented in the direction of the light source (as indicated by the arrow).
8. Press “Blank”, “Standard” or “Sample” softkey for measurement.



9. After measurement, remove the µCuvette from the cuvette shaft.
10. Use Kimwipes®, or lint-free tissue to wipe off the solution. Make sure solution on both arms of the µCuvette is removed.
11. The µCuvette is now ready for next measurement.

Eppendorf µCuvette G1.0 Problem Troubleshooting

Problems / Issues	Possible Causes	Ways to Troubleshoot
1a. Spectra demonstrates multiple tiny peaks. 1b. Sample shows higher concentration than expected. 1c. Sample displays very high A260/A280 purity ratio value (>3.0). 1d. Sample shows unusual high background value at 320 nm.	Non-formation of liquid column due to sample nature, insufficient sample loaded, pipetting error, or the arms of the µCuvette are not closed securely.	• Load more volume, especially for samples of sticky nature and when working with protein samples. • Check the hydrophobicity of the black coating on both arms by loading a drop of water onto it and observe if the water droplet beads up or stays flat. • Inaccurate sample volume pipetted. Check for secure tip attachment to the pipette and practice good pipetting technique when pipetting small volume.
2. Inconsistent measurement results.	a) Presence of bubbles, dust, turbidity, and air particles in the sample. b) Different side of the µCuvette arm is positioned facing the light source during blank and sample measurement respectively. c) Sample loading area is contaminated. d) The cuvette is used on other brand of spectrometer.	a) Activate background correction at 320 nm. Remove bubbles from sample and/or purify sample. b) Make sure the same side of the µCuvette arm is positioned facing the light source during blank and sample measurements. c) Clean the sample loading area as recommended above. d) The µCuvette is optimized to work with Eppendorf BioPhotometer, Eppendorf BioPhotometer plus, Eppendorf BioPhotometer D30, and Eppendorf BioSpectrometer.
3. Measurement result could not be interpreted.	Sample concentration detected is out of µCuvette detection range: ssDNA : 18.5 – 1110 ng/µL dsDNA : 25 – 1500 ng/µL RNA : 20 – 1200 ng/µL	Perform a sample dilution.
4. Sample measurement gave negative results.	The absorbance value of the blank solution is too high due to: a) Impurities in the blank solution or on the optical parts. b) Liquid column is not formed during blank measurement. c) Blank and sample solutions are mixed up.	a) Prepare a fresh blank solution and clean the quartz part of the µCuvette with fiber-free tissue, i.e. Kimwipes. b) Carry out visual examination of the liquid column formation on the µCuvette. c) Ensure the correct blank solution is loaded onto the µCuvette for blank measurement.
5. Measurement result is unusually low.	Incorrect selection of light path length of the cuvette during parameter setting (i.e., 10 mm path length is selected on instrument but 1 mm path length is used instead).	Define the correct setting for light path length as “1 mm”. Otherwise, the instrument produces 10-fold lower concentration.

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