Nucleic Acid Photometry

Check of critical parameters

Photometry Workflow – Evaluation of results

1. Linear absorbance range A_{260}

Optimum 0.1 – 1 A*

*optimal absorbance depends on technical specification of device

2. Background A_{320}

Optimum 0.0 A

(background correction ≤ 0.03 A)

3. RatioA_{260}/A_{280}

Optimum DNA 1.8 - 1.9
Optimum RNA 1.9 - 2.0

4. RatioA_{260}/A_{230}

Optimum > 2.0
Optimum < 2.5

Absorbance spectra of moleculs

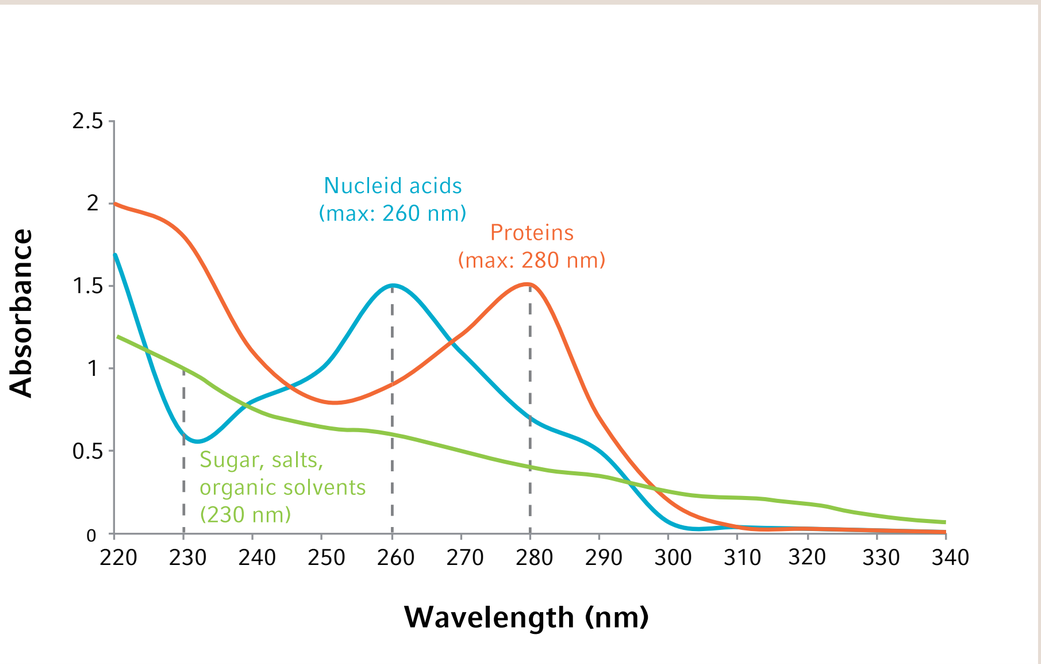


Figure 1: Absorbance spectrum of nucleic acids and proteins showing that you can easily detect contaminations by checking the ratios at 260/280 and 260/230 nm.

Absorbance spectra of pure and contaminated DNA

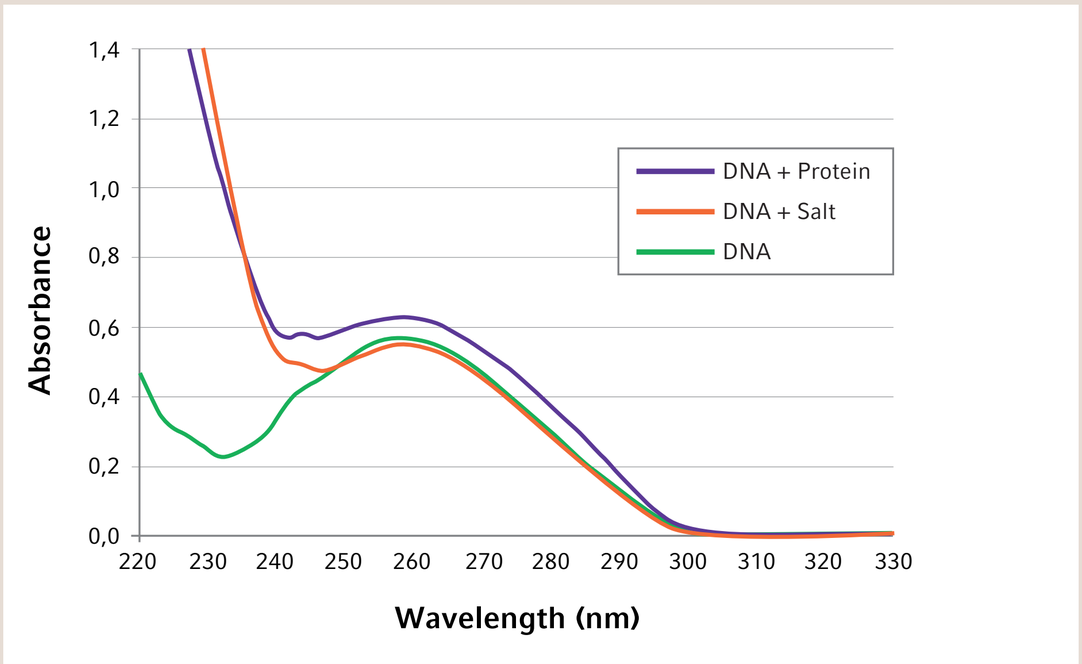


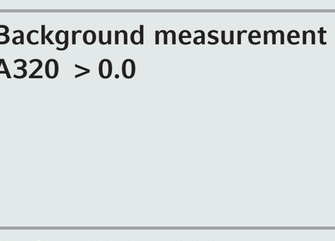
Figure 2: Absorbance spectrum of pure DNA and contaminated DNA showing that contamination has also an impact on the concentration of the measured nucleic acid.

Troubleshooting

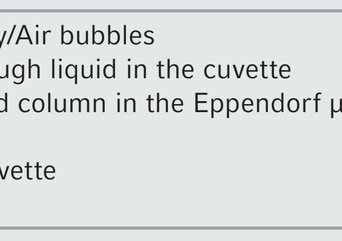
Problem	Reason	Solution
Absorbance at 260 nm < 0.05**	> Sample concentration too low	⇨ Use a cuvette with longer path length (if possible) ⇨ Concentrate your sample, or if it is a diluted sample, decrease the dilution ⇨ For very low concentrated samples use fluorescence spectrometry
> 2.0**	> Sample concentration is too high	⇨ Use a cuvette with shorter path length, like the Eppendorf µCuvette® G1.0 ⇨ Dilute your sample
Absorbance at 260 nm measuring range of device	> Inappropriate cuvette	⇨ Use UV-transparent cuvette ⇨ Ensure cuvette has correct light beam hight ⇨ Ensure cuvette is entered in the correct direction
Background measurement A320 > 0.0	> Turbidity/Air bubbles > Not enough liquid in the cuvette > No liquid column in the Eppendorf µCuvette® G1.0 > Dirty cuvette	⇨ Purify your sample ⇨ Remove air bubbles (pipette sample carefully into cuvette) ⇨ Ensure minimum required sample volume recommended of cuvette is used ⇨ Use more sample ⇨ Background correction if A is ≤ 0.03
Ratio A260/ A280 < 1.8 (for DNA) < 1.9 (for RNA)	> Contamination with proteins > Contamination with phenol or other aromatic compounds	⇨ Purify your sample
Ratio A260/ A280 > 1.9 (for DNA) > 2.0 (for RNA)	> Inappropriate blank solution	⇨ Use the same neutral, or alkaline buffer (e.g. TE-Buffer) for blank and sample
Ratio A260/ A230 < 2.0	> Contamination with proteins > Contamination with aromatic compounds, organic solvents, carbohydrates, salts > Inappropriate blank solution	⇨ Purify your sample
A 260 / 230 ratio > 2.5	> Inappropriate blank solution	⇨ Choose appropriate buffer (the same for blank and sample)

**Valid for the measuring range of the Eppendorf BioPhotometer® D30 and the Eppendorf BioSpectrometer®

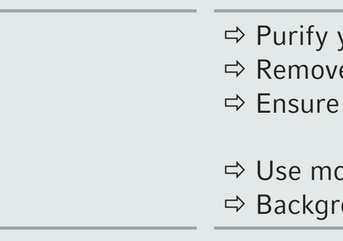
Photometry products



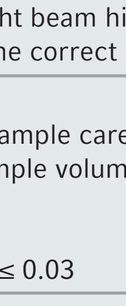
Eppendorf BioSpectrometer® basic



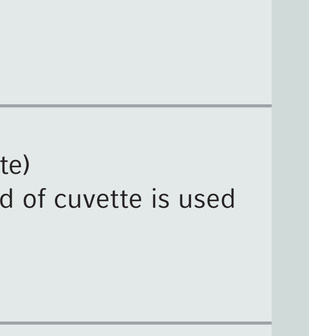
Eppendorf BioSpectrometer® flourescence



Eppendorf BioPhotometer® D30



Eppendorf µCuvette® G1.0



Eppendorf UVette®

Userguide 13 provides more information. Additional Application Notes and White Paper can be found online at www.eppendorf.com
Check out the Eppendorf Cuvette Navigator on www.eppendorf.com