

## Nucleic Acid Photometry

**Check of critical parameters** 

Photometry workflow – evaluation of results

1. Linear absorbance range A260

Optimum 0.1 – 1 A\*

\*optimal absorbance depends on technical specification of device

2. Background A320

Optimum 0.0 A

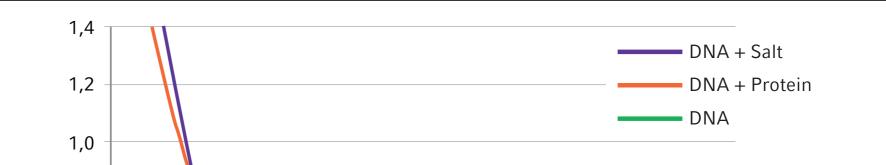
(background correction  $\leq$  0.03 A)

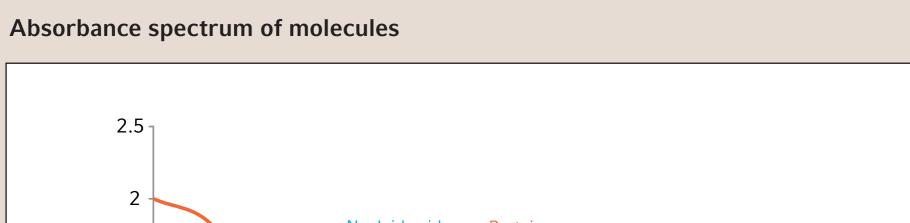
3. Ratio A260/A280

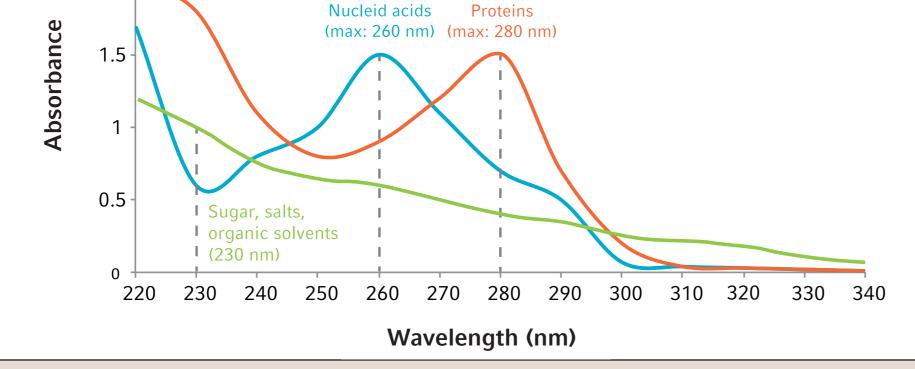
Optimum DNA 1.8 - 1.9 Optimum RNA 1.9 - 2.0 4. Ratio A260/A230

Optimum > 2.0 Optimum < 2.5

Absorbance spectrum of pure and contaminated DNA

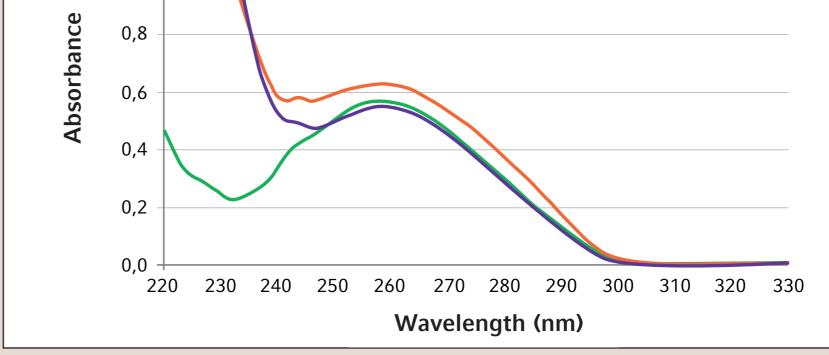






**Figure 1**: Absorbance spectrum of nucleic acids and proteins showing that you can easily detect contaminations by checking the ratios at 260/280 and 260/230 nm.

## Troubleshooting



**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.

Problem	Reason	Solution
Absorbance at 260 nm < 0.05**	Sample concentration too low	<ul> <li>⇒ Use a cuvette with longer path length (if possible)</li> <li>⇒ Concentrate your sample, or if it is a diluted sample, decrease the dilution</li> <li>⇒ For very low concentrated samples use fluorescence spectrometry</li> </ul>
> 2.0**	Sample concentration is too high	⇒ Use a cuvette with shorter path length, like the Eppendorf µCuvette <sup>®</sup> G1.0 ⇒ Dilute your sample
Measured absorbance in the UV range is above technical absorbance limit of the device	Inappropriate cuvette	<ul> <li>⇒ Use UV-transparent cuvette</li> <li>⇒ Ensure cuvette has correct light beam height</li> <li>⇒ Ensure cuvette is entered in the correct direction</li> </ul>
Background measurement A320 > 0.0	Turbidity / Air bubbles	<ul> <li>⇒ Purify your sample</li> <li>⇒ Remove air bubbles (pipette sample carefully into cuvette)</li> <li>⇒ Ensure minimum required sample volume for the cuvette is used</li> <li>⇒ Ensure there are no fingerprints on the optical surfaces of the cuvette</li> </ul>
	Not enough liquid in the cuvette Dirty cuvette	
		$\Rightarrow$ Background correction if A is $\leq 0.03$
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)	Inappropriate blank solution	$\Rightarrow$ Use the same neutral or alkaline buffer (e.g. TE-Buffer) for blank and sample

## > 2.0 (for RNA)

J. TE-Buffer) for blank and sample
g. TE-Buffer) for blank and sample
e.g

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



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