

04_01 Spectralphotometer NanoDrop

DIENSTAG, 18.5.2021

Goal-Setting

- The NanoDrop spectralphotometer is an instrument for nucleic acid and protein concentration measurements. The spectralphotometer is located in the Extension Lab on the left of our iGEM bench

Terms / abbreviations

- None

Risk areas

- None

Required materials and / or information

- Solution of blank e.g. MilliQ water or the solution without substance of interest
- 1 μ L of sample
- Pipettes, Eppendorf
- Tissue

Templates, devices, software

- NanoDrop spectralphotometer, ThermoScientific NanoDrop 2000
- NanoDrop Software

Preliminary work

- None

Operation

- Open the NanoDrop software on computer
- Choose the setting e.g. nucleic acid and ssDNA
- Open the lid, wipe the pedestal with a tissue to make sure the pedestal is clean and dry
- Blank the solution of the sample (whatever is in the solution, what is not the substance of interest)
- Apply 1 μ L on the pedestal, close the lid and press "blank" to measure
- Once the solution is blanked, wipe the pedestal, apply 1 μ L of the sample for each measurement
- Record results in following table:

Example: Recording NanoDrop results

	A	B	C	D	E	F	G	H	I	J
1	Sample name and type	Blank	Nucleic acid concentration	Unit	A260	A280	A260/A280 (1.8-2.0)	A260/230 (≥ 2.0)	Estimated length [nt]	Calculated concentration [nM]
2	P100 1:100 (09.06.), ssDNA	MilliQ	54.1	ng/ μ L	1.640	0.835	1.69	3.37	100	833.59

- Website to calculate the ng/ μ L into nM or μ M: [Weight to Molar Quantity \(for nucleic acids\) \(bioline.com\)](https://www.bioline.com/weight-to-molar-quantity-for-nucleic-acids/)

Weight to Molar Quantity (for nucleic acids)

This is used to convert the weight (weight concentration) into the molar quantity (molar concentration) for nucleic acids, and vice versa.

Help **Reset**

dsDNA Size of the nucl. acid [kb]: Put the number of kb here, e.g.
for the AT-rich primer it is 1

"weight" -> "mole":
µg Calculate mol molecules

"mole" -> "weight":
nmol Calculate g molecules

Calculation of the molar concentration:

1 µg In: 1 µl
Calculate M

Calculation of the weight from the molar concentration:

1 mM In: 1 µl
Calculate g

Troubleshooting

- The NanoDrop does not differentiate between nucleotides and nucleic acid strands
- To verify that the blank is fine or to determine the deviation, measure the blank as a sample
- For a pure DNA sample $A_{260}/A_{280} = 1.8-2.0$
 - Else: Contamination with proteins
- For a pure DNA sample $A_{260}/A_{230} \geq 2.0$
 - Else: Contamination with sugars, salts or phenols

Sources

- https://www.eppendorf.com/product-media/doc/de/59828/Eppendorf_Detection_Application-Note_279_BioPhotometer-D30_Detection-contamination-DNA-protein-samples-photometric-measurements.pdf

Follow-up work

- 03_04 PCR for ssDNA Samples