

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 μL 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 μL on the pedestal, close the lid and press "blank" to measure
- Once the solution is blanked, wipe the pedestal, apply 1 μL 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/ μL	1.640	0.835	1.69	3.37	100	833.59

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

Website for calculating molar quantity

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.

Put the number of kb here, e.g. for the AT-rich primer it is 0.1

"weight" --> "mole":

 molecules

"mole" --> "weight":

 molecules

Calculation of the molar concentration:

Set this to ng and type in the measured value from the nanodrop

Calculation of the weight from the molar concentration:

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