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 sprectralphotometer, ThermoScientific NanoDrop 2000
- NanoDrop Software

Preliminary work

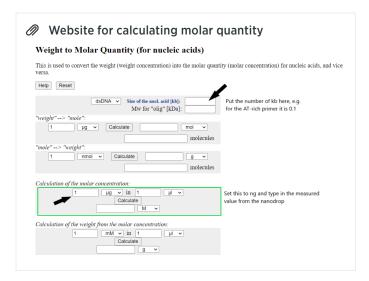
None

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										
	Α	В	С	D	Е	F	G	Н	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)



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 $A_{260}/A_{280} = 1.8-2.0$
 - o Else: Contamination with proteins
- For a pure DNA sample $A_{260}/A_{230} \ge 2.0$
 - o 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