WCSB, Wageningen University	IBISBA-SOP-WU25
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: *DNA and RNA concentration measurement*

distribution list			
changes to prior version:			
	name	signature	date
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Instruction

DNA and RNA concentration measurement

1. Introduction / Purpose

This protocol explains how to measure the concentration of DNA or RNA by use of a Nanodrop machine or a Qubit kit.

Keywords: DNA – RNA – concentration

2. Equipment and chemicals

2.1. Equipment

- Microvolume spectrophotometer (NanoDrop or other model)
- Fluorometer (Qubit or other brand)

2.2. Chemicals

- Qubit dsDNA BR Assay Kit by Thermo Fisher Scientific
  OR
- Qubit RNA BR Assay Kit by Thermo Fisher Scientific

2.4. Other materials

S*pecial consumables*

- For Qubit kits: thin-wall 0.5 ml PCR tubes
- For microvolume spectrophotometer: dust-free tissue (Kimcare or other brand)

3. Procedures

Microvolume spectrophotometer

- Clean the pedestal and the lid with water before use.
- Wipe pedestal and lid dry with special Kimcare tissue, repeat for each sample .
- Follow instructions for measuring of DNA or RNA as provided with the machine.

Qubit dsDNA or RNA BR assay kits

- The Qubit kits are used to measure DNA or RNA concentration very accurately with a fluorometer.
- On the website of Thermo Fisher Scientific elaborate manuals are published. On the next two pages a short version is shown.
- Ask your supervisor or a technician how to operate the fluorometer.

Qubit™ Assays

www.invitrogen.com/qubit

NOTE: For best results, store the dye and the buffer at room temperature. Store the DNA, RNA, and protein standards at 4°C. Ensure that all assay reagents are at **room temperature** before you begin.

1. Set up two Assay Tubes for the standards (three for the protein assay) and one tube for each user sample.

2. Prepare the Qubit™ **Working Solution** by diluting the Qubit™ reagent 1:200 in Qubit™ buffer. Prepare 200 µL of **Working Solution** for each standard and sample.

3. Prepare the Assay Tubes* according to the table below.

	Standard Assay Tubes	**User Sample** Assay Tubes
Volume of **Working Solution** (from step 2) to add	190 µL	180–199 µL
Volume of **Standard** (from kit) to add	10 µL	—
Volume of **User Sample** to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

* Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (set of 500, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

4. Vortex all tubes for 2–3 seconds.

5. Incubate the tubes for 2 minutes at room temperature (15 minutes for the Qubit™ protein assay).

6. Insert the tubes in the Qubit® 2.0 Fluorometer and take readings. For detailed instructions, refer to the Qubit® 2.0 Fluorometer manual.

7. *Optional:* Using the Dilution Calculator feature of the Qubit® 2.0 Fluorometer, determine the stock concentration of your original sample.

4. Remarks / troubleshooting

- A microvolume spectrophotometer can be used to quickly measure the concentration of DNA or RNA. However, the measurement is not always correct because contamination (RNA in a DNA sample or *vice versa*, or proteins, etc.) can distort the measurements.
- Qubit kit measurements are more accurate than microvolume spectrophotometers, but cost more time to perform. They are more accurate because the reagents only react with DNA or RNA.

6. Biosafety

No biosafety issues are associated with this method.

7. Acknowledgements

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