ENVIRONMENTAL GENOMICS LABORATORY

STANDARD OPERATING PROTOCOL · EnGeL-MOLB-NANODROP

Nucleic acid purity with the NanoDrop One spectrophotometer

Protocol ID	EnGeL-MOLB-NANODROP
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Area	Molecular biology (MOLB)
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Licence	Text: CC BY 4.0 · Code: MIT
Main hazards	None specific to this protocol

1 Purpose and scope

This protocol measures the purity of DNA extracts and PCR products with the NanoDrop One: the absorbance ratios A_{260}/A_{280} and A_{260}/A_{230}. It is used on extracts from EnGeL-MOLB-DNA-MAG, -DNA-COL, -DNA-BARC and -DNA-BEE, and on RNA from EnGeL-MOLB-RNA. NanoDrop concentrations are not used for DNA amounts: for those use fluorometry (EnGeL-MOLB-QUBIT for single samples, EnGeL-MOLB-PICOGREEN for 96-well plates).

2 Principle

The spectrophotometer measures the absorbance of a 1.5 µL drop at 230, 260 and 280 nm. Nucleic acids absorb most at 260 nm, proteins at 280 nm, and salts, phenol, guanidine and carbohydrates around 230 nm, so the ratios show carry-over from the extraction. The absorbance at 260 nm also includes RNA, single-stranded DNA and free nucleotides, which is why NanoDrop concentrations overestimate double-stranded DNA.

3 Before you start

Item	Details
Hands-on time	[TO CONFIRM]
Sample volume	1.5 µL per measurement
Prepare in advance	The blank: the last reagent of the extraction protocol (the buffer the samples are in)
Key safety points	See Safety and waste

4 Materials

4.1 Equipment

- NanoDrop One spectrophotometer (Thermo Scientific)
- Pipettes (0.5–10 µL) and filter tips

4.2 Consumables

- Lint-free laboratory wipes

4.3 Reagents and kits

- Nuclease-free water (cleaning)
- Blank: the last reagent used in the extraction protocol (e.g. elution buffer)

5 Reagent preparation

Not applicable.

6 Procedure

6.1 Before starting

- Use filter tips.
- Mix each sample gently and spin it down before measuring.

6.2 Measurement

1. Turn on the NanoDrop and let the initialisation run normally.
2. Clean the pedestal with 7 µL nuclease-free water.
3. Select the program for the sample type (dsDNA for DNA extracts and PCR products; RNA for RNA).
4. Load 1.5 µL of blank (the last reagent used in the extraction protocol), close the arm to measure it, then wipe the pedestal.
5. Load 1.5 µL of sample, close the arm to measure it, then wipe the pedestal. Repeat for each sample.
6. After all measurements, clean the pedestal with 7 µL nuclease-free water.

6.3 After finishing

- Clean the bench with 70% ethanol.
- Empty the small bench bin into the right waste bin.
- Wash your hands.
- Turn off equipment and lights.
- Lock doors, fridge and freezer.

7 Calculations

Not applicable: the instrument reports the ratios and the concentration (ng/µL).

8 Quality control

Control	Purpose	Acceptance criterion
Blank	Zero on the sample matrix	Measured before the samples, with the buffer the samples are in
DNA purity (A_{260}/A_{280}, A_{260}/A_{230})	Protein, salt or organic carry-over	Ideal: A_{260}/A_{280} 1.8–2.0 and A_{260}/A_{230} 2.0–2.2. Values outside are flagged, not failed: the PCR decides
Extraction blanks	Contamination during extraction	< 5 ng/µL (regarded as noise); also no band after PCR (extraction protocols)
RNA purity	Carry-over in RNA extracts	Classic ratios: A_{260}/A_{280} about 2.0 and A_{260}/A_{230} 2.0–2.2; values outside are flagged
RNA concentration	Enough RNA for downstream use	> 10 ng/µL

9 Data and metadata

- **Outputs:** A_{260}/A_{280}, A_{260}/A_{230} and the NanoDrop concentration (ng/µL) for each sample.
- **Where:** the *Sample_Tracking* sheet of the Metabarcoding Lab Pipeline workbook (A260/280, A260/230) or the project's records, and the lab book; instrument use in the NanoDrop logbook.

Minimum to record

- EnGeL sample label; date; operator
- Program used (dsDNA or RNA) and the blank
- A_{260}/A_{280}, A_{260}/A_{230} and the concentration, marked as NanoDrop values

10 Safety and waste

Follow the manufacturer's instructions and safety data sheets for each reagent. Biological samples are potentially hazardous and are handled following the facility's procedures. EnGeL lab rules:

- Before you enter: wear your lab coat, use appropriate gloves, closed shoes only, tie back long hair.
- Not allowed: eating or drinking; touching phones or laptops with contaminated gloves; bags or coats on benches.
- Label all reagents (name, concentration, date) and treat all chemicals as hazardous.

Waste

EnGeL waste streams (choose the right bin):

- **Biological solid waste** (black bin, white bag): contaminated gloves, contaminated disposable materials, solid biological laboratory waste, contaminated PPE. No liquids or sharps.
- **Specific hazardous waste** (red bin, red bag): chemicals and pharmaceuticals designated for this waste stream, specific hazardous laboratory waste, anatomical material; sharps only in a rigid sharps container. No loose sharps.
- **Biological liquid waste** (jerrican, UN 3291): liquid biological waste and contaminated biological liquids only. Use a funnel; rinse the original container and the funnel with dH_2O and discard the rinse water in the same jerrican.

For this protocol: tips and wipes go to biological solid waste (black bin).

11 Troubleshooting

Problem	Likely cause	Solution
Reading unstable or ratios odd	Dirty pedestal or bubble in the drop	Clean the pedestal with nuclease-free water; load the drop again without bubbles.
Low A_{260}/A_{230} in all samples	Wrong blank, or carry-over of extraction buffers	Blank with the buffer the samples are in; check the wash steps of the extraction.
Concentration much higher than by fluorometry	RNA, single-stranded DNA or nucleotides	Expected: use Qubit or PicoGreen for DNA amounts.

12 Remarks

For DNA amounts (library preparation, normalisation) always use fluorometry; NanoDrop is for purity.

13 References

Thermo Fisher Scientific. NanoDrop One user guide [TO CONFIRM: document version] (kept with the lab's manuals).

14 Version history and citation

Version	Date	Change	By
1.0.0	2026-11-20	First version as a separate protocol, split from EnGeL-MOLB-NAQC 1.3.0 (NanoDrop part, from the NanoDrop section of the EnGeL protocol list, 2024) at the request of the lab technicians.	Luís Cunha
1.1.0	2026-11-20	RNA criteria confirmed by Luís Cunha (classic ratios; > 10 ng/µL); Luísa Fraga: testing and adjustments.	Luís Cunha

How to cite

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15 Open questions

1. Hands-on time.
2. NanoDrop One user guide version; DOI.