STANDARD OPERATING PROTOCOL · EnGeL-MOLB-QUBIT

Double-stranded DNA concentration with the Qubit 4 fluorometer (dsDNA HS and BR assays)

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Authors	Diogo Fonseca (lead; Qubit protocol; ORCID 0009-0003-2564-7572); Andreia Quaresma (checking and formatting; ORCID 0000-0002-8678-5800); Luísa Fraga (testing and adjustments; ORCID 0000-0003-3153-4593); Luís Cunha (review and approval; ORCID 0000-0002-5870-2537)
Reviewed and approved by	Luís Cunha
Laboratory	Environmental Genomics Laboratory (EnGeL-UC), Environmental Ecology & Ecotoxicology Group (E3G), Centre for Functional Ecology, Department of Life Sciences, University of Coimbra · Laboratório Associado TERRA
Contact	luis.cunha@uc.pt · ewormuc@gmail.com
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Main hazards	Qubit reagent (dye in DMSO): handle with gloves [TO CONFIRM against the SDS]

1 Purpose and scope

This protocol measures the concentration of double-stranded DNA in single samples (DNA extracts, PCR products, libraries) with the Qubit 4 fluorometer and the dsDNA High Sensitivity (HS) assay, or the Broad Range (BR) assay for concentrated samples. For whole 96-well plates use EnGeL-MOLB-PICOGREEN; for purity use EnGeL-MOLB-NANODROP.

2 Principle

The Qubit dye fluoresces only when bound to double-stranded DNA. The instrument is calibrated with two standards of known concentration and converts the fluorescence of each sample into a concentration. Because the dye is specific to dsDNA, RNA, single-stranded DNA and free nucleotides are not counted, unlike in absorbance measurements.

3 Before you start

Item	Details
Hands-on time	[TO CONFIRM]
Sample volume	1 µL as standard; 1–20 µL is allowed, with working solution to a total of 200 µL (e.g. 5 µL sample + 195 µL), for dilute samples
Prepare in advance	Kit components at room temperature, as stated in the kit user guide; working solution for all tubes (standards + samples)
Key safety points	See Safety and waste

4 Materials

4.1 Equipment

- Qubit 4 Fluorometer (Invitrogen)
- Vortex mixer
- Pipettes and filter tips

4.2 Consumables

- Qubit assay tubes (0.5 mL, thin-walled, clear)

4.3 Reagents and kits

- Qubit dsDNA HS Assay Kit (high sensitivity): working solution and DNA standards #1 and #2 [TO CONFIRM: kit (1X or reagent + buffer) and catalogue number]
- Qubit dsDNA BR Assay Kit (broad range), for concentrated samples [TO CONFIRM: kit and catalogue number]

5 Reagent preparation

- **Working solution:** enough for all standards and samples (190 µL for each standard tube; 200 µL minus the sample volume for each sample tube).
  - **Standard HS or BR kits:** dilute the dye stock (in DMSO) 1:200 in the kit buffer (e.g. 5 µL dye + 995 µL buffer for five tubes), in a plastic tube; use within 3 h.
  - **1X kits:** the working solution is supplied ready to use (stable for 6 months after receipt); use it straight from the bottle.

6 Procedure

6.1 Before starting

- Use filter tips.
- Use only Qubit assay tubes, and label them on the lid, never on the side.

6.2 Measurement

1. Prepare the two standards in Qubit assay tubes: 190 µL working solution + 10 µL DNA standard #1; 190 µL working solution + 10 µL DNA standard #2.
2. For each sample, prepare a Qubit assay tube with 199 µL working solution + 1 µL sample.
3. Vortex vigorously for 3–5 s without creating bubbles.
4. Incubate all tubes at room temperature for 2 min.
5. On the Home screen, touch *dsDNA*, select *dsDNA High Sensitivity* (or *dsDNA Broad Range* with the BR kit) and touch *Read standards*.
6. Insert Standard #1, close the lid and touch *Read standard*; when the reading is complete (c. 3 s), remove it. Repeat with Standard #2.
7. Touch *Run samples*, select the sample volume (1 µL) and the output units.

8. Insert each sample tube, close the lid and select *Read tube*; remove the tube when the reading is complete (c. 3 s). Repeat for all samples.

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: with the sample volume entered, the instrument reports the concentration of the original sample (ng/µL).

8 Quality control

Control	Purpose	Acceptance criterion
Standards #1 and #2	Calibration	Accepted by the instrument, as described in the kit user guide; if not, prepare new standard tubes and calibrate again
Sample within the assay range	Valid reading	Above the range: dilute (or use the BR assay) and measure again. Below the range: report as below the detection limit
DNA concentration of extracts	Enough DNA for PCR	General rule: ≥ 10 ng/µL success; < 5 ng/µL fail; depends on the starting quantity and parent material; between 5 and 10 ng/µL the PCR result decides

9 Data and metadata

- **Outputs:** dsDNA concentration (ng/µL) per sample.
- **Where:** the *Sample_Tracking* sheet of the Metabarcoding Lab Pipeline workbook (DNA_ngul) or the project's records, and the lab book; instrument use in the QC user log.

Minimum to record

- EnGeL sample label; date; operator
- Assay (dsDNA HS or BR) and kit lot; sample volume and any dilution
- Concentration (ng/µL), marked as Qubit 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.

- Qubit reagent: the dye is dissolved in DMSO, which passes through skin; wear gloves [TO CONFIRM: hazards from the SDS].

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: assay tubes and tips go to biological solid waste (black bin) [TO CONFIRM].

11 Troubleshooting

Problem	Likely cause	Solution
"Out of range" reading	Sample too concentrated or too dilute for the HS assay	Dilute the sample, or use more sample volume (and enter it); for high concentrations use the BR assay.
Readings drift	Tubes warmed in the hand, or read long after mixing	Hold tubes by the lid; read within the time given in the kit instructions.
Standards fail	Wrong tubes or working solution	Use Qubit assay tubes; prepare fresh working solution.

12 Remarks

Qubit concentrations are for double-stranded DNA only and are the values to use for normalisation and library preparation.

Assay ranges (amount of DNA in the 200 µL assay tube; Thermo Fisher technical note on the Qubit 1X dsDNA assays):

Assay	Core range (high confidence)	Extended range (moderate confidence)
dsDNA HS (standard and 1X)	0.2–100 ng	0.1–0.2 ng and 100–120 ng
dsDNA BR (standard kit)	4–1 000 ng	1 000–2 000 ng
dsDNA BR (1X kit)	4–2 000 ng	2 000–4 000 ng

With 1 µL of sample, the HS core range corresponds to 0.2–100 ng/µL in the sample. Above the core range, dilute the sample or use the BR assay.

13 References

Thermo Fisher Scientific. Qubit dsDNA HS and BR Assay Kits, user guides [TO CONFIRM: document and revision] (kept with the lab's kit manuals).

Thermo Fisher Scientific (Invitrogen). Qubit 1X dsDNA assays: simplified workflow and improved performance. Technical note (kept with the lab's kit 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 (Qubit part, from the Qubit section of the EnGeL protocol list, 2024) at the request of the lab technicians.	Luís Cunha
1.1.0	2026-11-20	Qubit 4 (from the instrument photo); BR assay also used; readings above the range diluted and repeated (confirmed by Luís Cunha); Luísa Fraga: testing and adjustments.	Luís Cunha
1.2.0	2026-11-20	Kit preparation, sample volume (1–20 µL), standards and assay ranges from the Qubit user guidance (as requested by Luís Cunha).	Luís Cunha

How to cite

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15 Open questions

1. Which kits the lab holds (standard or 1X; HS and BR) and their catalogue numbers.
2. Hands-on time; hazards from the SDS; waste route; DOI.