



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L213

Qubit 4 Fluorometer

Standard Operating Procedure

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Invitrogen Qubit 4 Fluorometer. The Qubit uses fluorescent dyes that bind selectively to the target molecule (dsDNA, ssDNA, RNA, or protein), providing more accurate quantification than UV absorbance methods, especially at low concentrations or when contaminants are present. For the role of Qubit measurements within the DNA extraction workflow, see [SOP-L700 DNA Extraction](#).

2. Scope

- **Applies to:** All use of the Qubit 4 Fluorometer in the EA Program laboratory, including dsDNA Broad Range (BR), dsDNA High Sensitivity (HS), RNA, and protein assays
- **Does not apply to:** UV-Vis spectrophotometric quantification (see [SOP-L212](#)), Turner Designs fluorometry for chlorophyll or other environmental analytes (see [SOP-L203](#))
- **Location:** Instrument bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Maintain reagent inventory, schedule service
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Broad Range (BR) assay:** A Qubit assay kit designed for DNA concentrations of 2–1,000 ng/μL; less sensitive than the HS assay but covers a wider dynamic range; used when high DNA yield is expected.
- **dsDNA:** Double-stranded DNA; the form quantified by most Qubit DNA assay kits; the fluorescent dye binds specifically to dsDNA, not to ssDNA, RNA, or protein contaminants.
- **Fluorophore:** A fluorescent chemical compound that absorbs light at a specific excitation wavelength and emits light at a longer emission wavelength; the Qubit dye is a fluorophore that binds selectively to dsDNA, ssDNA, or RNA depending on the kit.
- **High Sensitivity (HS) assay:** A Qubit assay kit designed for DNA concentrations of 0.2–100 ng/μL; more sensitive than BR; preferred for low-yield environmental DNA extractions from soil or water.
- **Qubit assay tube:** A thin-walled 0.5 mL PCR tube in which the working solution and sample are mixed for measurement; must be free of fluorescent contaminants; do not substitute with other tube types.
- **Working solution:** A freshly prepared mixture of Qubit reagent diluted in buffer at a ratio specified by the kit (usually 1:200); prepared fresh for each session; stable for up to 3 hours at room temperature.

5. Safety and Hazards

Hazards

- **Chemical:** Qubit reagent concentrate contains DMSO. Avoid skin contact; review the SDS before handling.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

For chemical splashes, flush with water for at least 15 minutes and consult the SDS. For biological spills, clean per [SOP-L002 Laboratory Safety](#).

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Qubit 4 Fluorometer	Instrument bench, SGM 133	Invitrogen / Thermo Fisher
USB drive or Wi-Fi connection	–	For data export

6.2. Reagents and Consumables

Item	Catalog No.	Storage
Qubit dsDNA BR Assay Kit	Q32850	Room temperature
Qubit dsDNA HS Assay Kit	Q32851	Room temperature
Qubit assay tubes (0.5 mL, thin-wall)	Q32856	Room temperature
Nuclease-free water	Various	Room temperature

Note

Use only the thin-wall 0.5 mL Qubit assay tubes (Cat. Q32856). Standard microcentrifuge tubes are not optically compatible and will produce inaccurate readings.

7. Procedure

7.1. Selecting the Assay

Choose the assay based on expected concentration (Invitrogen, 2019):

Assay	Detection Range	When to Use
dsDNA BR	2–1,000 ng	Most soil/sediment extractions
dsDNA HS	0.2–100 ng	Low-yield samples (e.g., water filters, dilute extracts)
RNA BR / HS	See kit documentation	RNA quantification
Protein	See kit documentation	Protein quantification

7.2. Preparing the Working Solution

1. Calculate the volume of working solution needed: 200 uL per assay tube (all samples plus 2 standards)
2. Dilute the Qubit reagent concentrate 1:200 in Qubit buffer. For example, for 10 tubes: 10 uL reagent + 1,990 uL buffer = 2,000 uL total.
3. Vortex briefly to mix

! Important

Prepare the working solution fresh for each session. Do not store diluted working solution.

7.3. Preparing Standards

1. Label two Qubit assay tubes: **Std 1** and **Std 2**
2. Add 190 uL of working solution to each tube
3. Add 10 uL of Standard 1 to the first tube and 10 uL of Standard 2 to the second tube
4. Vortex each tube briefly (2–3 seconds)

7.4. Preparing Samples

1. Label one Qubit assay tube per sample
2. Add 198 uL of working solution to each tube
3. Add 2 uL of sample (DNA extract, RNA, or protein solution) to the corresponding tube
4. Vortex each tube briefly (2–3 seconds)

💡 Tip

The sample volume can range from 1–20 uL as long as the total volume in each tube equals 200 uL (adjust the working solution volume accordingly). Using 2 uL of sample minimizes

consumption of precious extracts.

7.5. Incubation

1. Incubate all tubes (standards and samples) at room temperature for 2 minutes (Invitrogen, 2019)
2. Do not incubate for longer than 30 minutes; fluorescence signal decays over time

7.6. Calibration

1. On the Qubit home screen, select the appropriate assay (e.g., **dsDNA Broad Range**)
2. Tap **Read Standards**
3. Insert the Std 1 tube, close the lid, and tap **Read**
4. When prompted, insert the Std 2 tube, close the lid, and tap **Read**
5. Verify that the calibration is accepted (green checkmark). If it fails, prepare fresh standards and repeat.

7.7. Reading Samples

1. Tap **Run Samples**
2. Set the sample volume to match the volume of sample added (e.g., 2 uL)
3. Set the output units (ng/uL is standard for DNA extracts)
4. Insert the sample tube, close the lid, and tap **Read**
5. Record the concentration (ng/uL, corrected for dilution by the instrument)
6. Repeat for all samples

7.8. Data Export

1. Export results via USB or Wi-Fi to the designated project folder
2. Use a consistent file-naming convention: YYYY-MM-DD_Qubit_ProjectName.csv
3. Record results in the lab notebook and/or project data sheet

7.9. Shutdown and Daily Maintenance

1. Remove all tubes from the instrument
2. Wipe the sample chamber with a lint-free tissue if any liquid was spilled
3. Close the lid
4. Dispose of used assay tubes (see Section 8)

8. Quality Control and Acceptance Criteria

- Run fresh standards at the beginning of each session; do not rely on a previous calibration
- Standards must be within the acceptable range indicated by the instrument (green checkmark)

- If a sample reads “Out of Range,” re-prepare it using the other assay sensitivity (e.g., switch from BR to HS for low-concentration samples)
- Acceptable DNA yield from 0.25 g of soil: typically 1–20 ng/uL in a 100 uL elution, depending on soil type
- If the NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; trust the Qubit value for downstream planning

TBD – establish a periodic performance check using Qubit DNA standards of known concentration.

9. Waste Disposal

Waste Type	Disposal Method
Used Qubit assay tubes	Standard trash (rinse if biohazardous)
Leftover working solution	Standard drain (dilute DMSO, non-hazardous at this concentration)
Pipette tips	Standard trash (or biohazard if samples are hazardous)

10. References

Invitrogen. (2019). *Qubit 4 fluorometer user guide*. Thermo Fisher Scientific. <https://www.thermofisher.com/us/en/home/references/protocols/nucleic-acid-purification-and-analysis/qubit-assay-protocols.html>

11. Training Record

Before using the Qubit independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Observe a trained operator demonstrate standard preparation, calibration, sample reading, and data export
4. Perform a practice session (calibration plus at least 5 samples) under supervision
5. Be signed off by the PI or lab manager