STANDARD OPERATING PROTOCOL · EnGeL-MOLB-PICOGREEN

Double-stranded DNA concentration in 96-well plates with PicoGreen (FLUOstar Omega plate reader)

Protocol ID	EnGeL-MOLB-PICOGREEN
Version	1.1.1
Date	2026-11-20
Status	draft
Area	Molecular biology (MOLB)
Authors	Andreia Quaresma (lead; PicoGreen protocol; 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
Licence	Text: CC BY 4.0 · Code: MIT
Main hazards	PicoGreen reagent (in DMSO): handle with gloves [TO CONFIRM against the SDS]

1 Purpose and scope

This protocol measures the concentration of double-stranded DNA in many samples at once (DNA extracts, PCR products, libraries in 96-well format) with Quant-iT PicoGreen and the FLUOstar Omega plate reader. For single samples use EnGeL-MOLB-QUBIT; for purity use EnGeL-MOLB-NANODROP.

2 Principle

PicoGreen fluoresces strongly only when bound to double-stranded DNA. The fluorescence of each well is compared with a standard curve made from a dilution series of lambda DNA, so the result is specific to dsDNA. The lab uses half the volumes of the manufacturer's assay (100 µL per well) with the same high-range standards.

3 Before you start

Item	Details
Hands-on time	30 min for a 96-well plate

Item	Details
Total time	40 min
Sample volume	2 µL per well
Prepare in advance	1X TE and PicoGreen working reagent, fresh on the day, in the dark
Key safety points	See Safety and waste

4 Materials

4.1 Equipment

- Fluorescence plate reader FLUOstar Omega (BMG Labtech) with protocol “PicoGREEN_LC_100” (excitation filter 485-12, emission filter 520)
- Pipettes (single and multichannel) and filter tips; vortex mixer; plate shaker (optional)

4.2 Consumables

- Black flat-bottom 96-well plates (Greiner 96 F-bottom, as set in the reader protocol)
- Plastic tubes only (PicoGreen binds to glass)

4.3 Reagents and kits

- Nuclease-free water
- Quant-iT PicoGreen dsDNA reagent and 20X TE buffer (Invitrogen) [TO CONFIRM: catalogue number]
- Lambda DNA standard (100 µg/mL, supplied with the PicoGreen kit)

5 Reagent preparation

- **1X TE:** dilute 20X TE 1:20 in nuclease-free water (e.g. 2.5 mL 20X TE + 47.5 mL water for 50 mL).
- **Lambda working stock (2 ng/µL = 2 µg/mL):** dilute the 100 µg/mL lambda DNA 50-fold in 1X TE, e.g. 10 µL Lambda DNA + 490 µL 1X TE for 500 µL (the earlier lab version, 9 µL + 441 µL, gives 450 µL at the same concentration).
- **PicoGreen working reagent:** dilute 1:200 in 1X TE, fresh on the day, in the dark. Per 96-well plate, prepare 5 mL: 25 µL PicoGreen + 4 975 µL 1X TE (50 µL per well).

Standard curve (in duplicate)

PicoGreen standards.

Standard	Lambda working stock (µL)	1X TE (µL)	Working reagent (µL)	Concentration (ng/mL)
S1	50	0	50	1000
S2	5	45	50	100
S3	0.5	49.5	50	10
Blank	0	50	50	0

6 Procedure

6.1 Before starting

- Use filter tips at all steps; plastic tubes only.
- Keep the PicoGreen reagent and working reagent protected from light.

6.2 Plate and reading

1. Pipette each standard into the black plate in duplicate (table in Reagent preparation).

2. Samples: 2 µL sample + 48 µL 1X TE per well + 50 µL working reagent.
3. Mix by pipetting or a short plate shake.
4. Incubate for 2 min at room temperature, protected from light.
5. Plate reader: *Manage Protocol* → "PicoGREEN_LC_100"; *Start measurement* → *Gain Adjustment*; then *Start measurement*. The reader protocol is a fluorescence-intensity endpoint read (Greiner 96 F-bottom plate; excitation filter 485-12, emission filter 520; top optic; 30 flashes per well; settling time 0.5 s). The gain is set at 80–90% of the highest standard (1 000 ng/mL), and the blank is subtracted in the evaluation.
6. In the reader software, fit a linear regression of the standards (S1–S3 and blank) (*Calculation* → *Standard curve calculations* → *Linear regression fit*); then, in *Microplate View*, select *Linear Regression Fit* under *Recalculate concentration*.

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

Sample concentration

$$c_{\text{sample}} = \frac{c_{\text{well}} \times 50}{1000}$$

- c_{sample}: concentration of the sample (ng/µL)
- c_{well}: concentration read from the standard curve (ng/mL)
- 50: dilution of the sample in the well (2 µL in 100 µL; dimensionless)
- 1000: conversion from ng/mL to ng/µL

Worked example (illustrative value): a well reads 400 ng/mL → 400 × 50 / 1000 = 20 ng/µL.

8 Quality control

Control	Purpose	Acceptance criterion
Standard curve (S1–S3 + blank, in duplicate)	Calibration	$R^2 \geq 0.99$; if not, prepare the standards again and repeat the curve before reading the samples
Samples within the curve	Valid reading	Above S1 (1 000 ng/mL in the well): dilute in 1X TE and measure again. Below the lowest standard: report as below the curve
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 well; plate-reader export with the standard curve.
- **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

- Plate ID and plate map (EnGeL sample labels); date; operator
- PicoGreen kit lot; reader protocol used
- Standard curve R^2; concentrations (ng/µL), marked as PicoGreen 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.
- PicoGreen is dissolved in DMSO, which passes through skin, and binds DNA: wear gloves; keep away from light [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: plates, tubes and tips go to biological solid waste (black bin) [TO CONFIRM].

11 Troubleshooting

Problem	Likely cause	Solution
Standard curve $R^2 < 0.99$	Pipetting error in the standards, or old lambda working stock	Prepare the standards again and repeat the curve.
Low signal overall	Reagent exposed to light, or glass tubes used	Prepare the working reagent fresh, in the dark, in plastic tubes.
Samples above S1	Concentration beyond the curve	Dilute the samples in 1X TE and measure again; correct for the dilution.

12 Remarks

The manual uses a 200 µL assay (100 µL sample + 100 µL working reagent); the lab uses half volumes (100 µL), with the same high-range standards (1 µg/mL, 100 ng/mL, 10 ng/mL, blank). For very dilute samples the manual gives a low-range curve (25 ng/mL to 250 pg/mL).

13 References

Thermo Fisher Scientific (Invitrogen) (n.d.). Quant-iT PicoGreen dsDNA Reagent and Kits, user guide, Pub. No. MAN0001931, Rev. A.0.

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 (PicoGreen part, from Andreia Quaresma's "Quality Control: PicoGreen dsDNA quantification (96-well plate)" v1.0, 2026, checked against the Quant-iT PicoGreen user guide) at the request of the lab technicians.	Luís Cunha
1.1.0	2026-11-20	Samples above the standard curve diluted and measured again (confirmed by Luís Cunha); Luísa Fraga: testing and adjustments.	Luís Cunha
1.1.1	2026-11-20	Action when the standard curve fails: repeat the curve (confirmed by Luís Cunha).	Luís Cunha

How to cite

Quaresma A, Fraga L, Cunha L (2026). Double-stranded DNA concentration in 96-well plates with PicoGreen (FLUOstar Omega plate reader). EnGeL-UC protocol EnGeL-MOLB-PICOGREEN, version 1.1.1. Zenodo. DOI [TO CONFIRM].

Licence

Text: CC BY 4.0. Code: MIT. Logos are the property of their institutions and are not covered by the licence.

15 Open questions

1. Kit catalogue number; hazards from the SDS; waste route.
2. DOI.