



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L212

NanoDrop One Spectrophotometer

Standard Operating Procedure

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1. Purpose

This procedure describes the operation, calibration verification, and maintenance of the Thermo Scientific NanoDrop One micro-volume UV-Vis spectrophotometer. The NanoDrop measures nucleic acid and protein concentrations from samples as small as 1–2 uL and provides absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}) used to assess sample purity. For the role of NanoDrop measurements within the DNA extraction workflow, see [SOP-L700 DNA Extraction](#).

2. Scope

- **Applies to:** All use of the NanoDrop One spectrophotometer in the EA Program laboratory, including nucleic acid quantification, protein quantification (A_{280} method), and custom wavelength measurements
- **Does not apply to:** Cuvette-based spectrophotometers (see [SOP-L201](#)), fluorometric quantification (see [SOP-L213](#)), or Hach benchtop spectrophotometry (see [SOP-L303](#))
- **Location:** Instrument bench, Seaver ??

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule maintenance, manage software updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **A_{260} :** Absorbance at 260 nm; the wavelength at which double-stranded DNA and RNA absorb most strongly; used to quantify nucleic acid concentration.
- **A_{280} :** Absorbance at 280 nm; absorbed primarily by aromatic amino acids (tyrosine, tryptophan) and by phenolic contaminants; used to assess protein contamination in nucleic acid preparations.
- **A_{260}/A_{280} ratio:** The ratio of absorbance at 260 nm to 280 nm; pure dsDNA gives ~1.8; pure RNA gives ~2.0; values below 1.7 indicate protein or phenol contamination.
- **A_{260}/A_{230} ratio:** The ratio of absorbance at 260 nm to 230 nm; values of 2.0–2.2 are expected for pure nucleic acids; low values indicate contamination with chaotropic salts (guanidinium), carbohydrates, or humic acids.
- **Micro-volume spectrophotometry:** Absorbance measurement using 1–2 μL of sample held by surface tension between a pedestal and an optical fiber arm; eliminates the need for cuvettes and allows measurement of very small volumes.
- **Pedestal:** The fixed lower optic of the NanoDrop on which the sample droplet is placed; must be cleaned with a lint-free wipe between measurements to prevent carryover.
- **Pathlength normalization:** Automatic adjustment of the measured absorbance to a standardized 10 mm pathlength equivalent; allows direct comparison with traditional cuvette measurements.

5. Safety and Hazards

Hazards

- **Optical:** The NanoDrop uses a pulsed xenon flash lamp. Do not look directly into the light source when the pedestal arm is open.
- **Biological:** Samples may contain hazardous biological material. Treat all samples as potentially infectious.
- **Chemical:** Cleaning solvents (e.g., 0.5 M HCl for decontamination) require appropriate handling.

5.1. Required PPE

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

5.2. Emergency Procedures

For biological spills on or near the instrument, clean and disinfect per [SOP-L002 Laboratory Safety](#). If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
NanoDrop One spectrophotometer	Instrument bench, SGM 133	Thermo Scientific
Connected laptop or NanoDrop display	Instrument bench	For data export

6.2. Consumables

Item	Notes
Lint-free wipes (Kimtech)	For pedestal cleaning between samples
Nuclease-free water	For blanking and pedestal cleaning
Elution buffer	Must match the buffer used in extraction

7. Procedure

7.1. Startup

1. Open the NanoDrop One software (or wake the integrated touchscreen)
2. Select the appropriate application (e.g., **Nucleic Acid**, **Protein A280**, or **UV-Vis**)
3. Clean the upper and lower pedestals with 2 uL of nuclease-free water; wipe with a lint-free tissue
4. Perform a blank measurement (see Section 6.2)

7.2. Blanking

1. Pipette 2 uL of the blank solution onto the lower pedestal. The blank must match the sample matrix – use the same elution buffer or solvent that the samples are dissolved in.
2. Lower the sampling arm and tap **Blank**
3. Verify that the blank spectrum is flat and the baseline absorbance at 260 nm is near zero
4. Wipe both pedestals with a lint-free tissue



Tip

Re-blank whenever you switch sample types, change buffers, or if the instrument has been idle for more than 30 minutes.

7.3. Measuring Samples

1. Pipette 2 uL of sample onto the lower pedestal
2. Lower the sampling arm and tap **Measure**
3. Record the following for each sample:
 - Concentration (ng/uL)
 - A_{260}/A_{280} ratio
 - A_{260}/A_{230} ratio
4. Wipe both pedestals with a lint-free tissue between every sample
5. Repeat for all samples

7.4. Interpreting Results

Parameter	Expected Range	Interpretation
A_{260}/A_{280}	1.8–2.0	Values below 1.8 suggest protein or phenol contamination
A_{260}/A_{230}	2.0–2.2	Values below 2.0 suggest humic acid, carbohydrate, salt, or guanidine contamination

i Note

For environmental DNA extracts, A_{260}/A_{230} ratios are frequently below 2.0. If the NanoDrop concentration is more than twice the Qubit reading, the UV absorbance is likely inflated by co-extracted contaminants; use the Qubit value for planning downstream reactions.

7.5. Data Export

1. Save the session file (.tsv or .csv) to the designated project folder on the connected laptop
2. Use a consistent file-naming convention: YYYY-MM-DD_NanoDrop_ProjectName.tsv
3. Record results in the lab notebook and/or project data sheet

7.6. Shutdown and Daily Maintenance

1. Wipe both pedestals with 2 uL of nuclease-free water, then dry with a lint-free tissue
2. If protein or concentrated DNA residue is visible, clean with 2 uL of 0.5 M HCl, wait 10 seconds, then rinse with nuclease-free water
3. Close the software and lower the sampling arm
4. Cover the instrument with the dust cover if available

8. Quality Control and Acceptance Criteria

- Verify instrument performance at the start of each session by measuring a blank; the 260 nm absorbance should read 0.00 +/- 0.02
- 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
- *TBD – establish a schedule for periodic performance verification using Thermo Scientific NanoDrop verification standards*

9. Waste Disposal

Waste Type	Disposal Method
Used lint-free wipes	Standard trash
Pipette tips	Standard trash (or biohazard if samples are hazardous)
Residual sample on pedestal	Wiped and cleaned as part of shutdown

10. References

- Thermo Fisher Scientific. *NanoDrop One/One^C User Guide*. Thermo Fisher Scientific, Waltham, MA.

- Desjardins, P. & Bhatt, T. (2010). Micro-volume quantification of nucleic acids. *Current Protocols*, A.3J.1–A.3J.16.

11. Training Record

Before using the NanoDrop independently, personnel must:

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