



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L215

Hach DR3900 Benchtop Spectrophotometer

Standard Operating Procedure

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1. Purpose

This procedure describes the setup, operation, routine maintenance, and troubleshooting of the Hach DR3900 benchtop spectrophotometer as a laboratory instrument. The DR3900 uses a tungsten lamp (visible range) and a deuterium lamp (UV range) to measure absorbance and transmittance across 320–1100 nm, supporting both factory-stored programs for Hach reagent kits and user-defined wavelength methods (Hach Company, 2024a, 2024b).

For method-specific chemistry, reagent handling, and the analyte Method Registry (nutrients, metals, and general water quality parameters), see [SOP-L303 Hach Colorimetric Analysis](#). For field use of the portable Hach DR900 colorimeter, see [SOP-F208 Portable Spectrophotometer / Colorimeter \(Field\)](#).

2. Scope

- **Applies to:** All use of the Hach DR3900 benchtop spectrophotometer in the EA Program laboratory, including stored-program colorimetric analysis, user-defined single-wavelength measurements, wavelength scans, and time-course (kinetic) measurements
- **Does not apply to:** Micro-volume UV-Vis measurements (see [SOP-L212 NanoDrop](#)); fluorometric quantification (see [SOP-L203 Trilogy Fluorometer](#)); portable field colorimetry (see [SOP-F208](#)); general spectrophotometer theory (see [SOP-L201](#))
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, authorize instrument access
Lab Manager	Schedule lamp replacement and annual verification, manage firmware updates
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Absorbance (A):** The negative base-10 logarithm of transmittance: $A = -\log_{10}(T)$; directly proportional to analyte concentration (Beer-Lambert law) within the linear range of the instrument.
- **Beer-Lambert law:** The relationship between absorbance, path length, and concentration: $A = \varepsilon \cdot c \cdot l$, where ε is the molar absorptivity, c is concentration, and l is the optical path length (cell width); the basis for all colorimetric quantification.
- **Blank (reagent blank):** A sample containing all reagents except the analyte; used to zero the instrument and correct for absorbance contributed by the reagent matrix.
- **Colorimetric method:** An analytical technique in which a chemical reagent reacts with the analyte to produce a colored complex whose absorbance is proportional to analyte concentration.
- **TNT plus vial:** A Hach proprietary pre-dosed reagent vial for the DR3900; contains all required reagents sealed in a glass vial; provides consistent results without manual reagent preparation.
- **Transmittance (T):** The fraction of incident light that passes through the sample: $T = I/I_0$; related to absorbance by $A = -\log_{10}(T)$.
- **Wavelength scan:** A measurement mode in which the instrument records absorbance across a range of wavelengths to identify the absorption maximum of an unknown compound.

5. Safety and Hazards

Hazards

- **Optical:** The deuterium lamp emits ultraviolet radiation. Do not look directly into the sample compartment when the lid is open and the lamp is on. The instrument's interlock dims the lamp when the lid is raised, but UV exposure remains possible during diagnostic modes.
- **Chemical:** Sample solutions and reagents placed in the instrument may be hazardous. Refer to the relevant method SOP (e.g., [SOP-L303](#)) and the reagent SDS for handling precautions.
- **Electrical:** Do not operate the instrument with wet hands or near standing water. If liquid enters the instrument housing, disconnect power immediately and contact the lab manager.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (when handling reagents or samples)

5.2. Emergency Procedures

For chemical spills on or near the instrument, clean the spill per [SOP-L003 Hazardous Materials Handling](#) before resuming use. If liquid enters the instrument, unplug the power cord and do not attempt to power on until the lab manager has inspected the unit.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Hach DR3900 benchtop spectrophotometer	Lab bench, SGM 133	Wavelength range 320–1100 nm
Light shield / adapter	With DR3900	For 13 mm and 16 mm round vials (TNT plus)
USB cable or Ethernet connection	Lab bench	For data export to PC

6.2. Sample Cells

Cell Type	Path Length	Use
10 mm square glass or quartz	10 mm	General absorbance; user-defined methods
1-inch round glass	25.4 mm	Most Hach stored-program methods
16 mm round (TNT plus vials)	16 mm	TNT plus pre-dosed vial methods

6.3. Consumables

Item	Notes
Lint-free wipes (Kimwipes)	For cleaning sample cell exteriors
Silicone oil and applicator cloth	For 1-inch round cells (reduces light scattering at the glass surface)
DI water (Type II or better)	For blanking and rinsing

7. Procedure

7.1. Startup and Self-Check

1. Verify that the sample compartment is empty and the lid is closed
2. Turn on the DR3900 using the power switch on the rear panel
3. The instrument performs an automatic self-check, including lamp tests and wavelength verification. This takes approximately 30 seconds. Do not open the lid or insert samples until the home screen appears.

4. Allow the lamps to warm up for at least 15 minutes before making measurements that require high photometric accuracy (e.g., user-defined methods at low absorbance). For routine stored-program analyses, measurements may begin after the self-check completes.

7.2. Selecting an Operating Mode

From the home screen, select one of the following modes:

Mode	When to Use
Stored Programs	Pre-programmed Hach reagent kit methods; see SOP-L303 for the Method Registry
Single Wavelength	User-defined absorbance or %T at a specific wavelength
Multi Wavelength	Simultaneous measurement at 2–4 wavelengths
Wavelength Scan	Absorbance spectrum over a defined range
Time Course	Kinetic measurement at a fixed wavelength over time

7.3. Preparing and Inserting Sample Cells

1. **Select the correct cell type** for the method (see Section 5). Square cells must be oriented so the clear optical faces align with the light path (arrow on the cell pointing toward the detector).
2. **Fill the cell** to the level required by the method. Do not overfill; liquid on the outside of the cell causes erroneous readings.
3. **Clean the exterior** of the cell with a lint-free wipe. For 1-inch round cells, apply a thin film of silicone oil and wipe with the oiling cloth to eliminate fingerprints and water droplets (Hach Company, 2024a).
4. **Insert the cell** into the cell holder. Close the sample compartment lid fully before reading.

Cuvette Selection for UV Work

Standard glass and plastic cells absorb UV light below approximately 340 nm. For measurements below 340 nm in user-defined or wavelength scan modes, use quartz cells (Skoog et al., 2018).

7.4. Zeroing (Blanking) the Instrument

1. Fill a clean cell with the blank solution. The blank must match the sample matrix: DI water for simple aqueous samples, or the reagent blank specified by the Hach method.
2. Insert the blank cell and close the lid
3. Press **Zero** (stored-program mode) or **Zero/Blank** (single wavelength mode)
4. Verify that the display reads 0.000 Abs (or 100.0 %T)
5. Remove the blank cell

i Note

Re-zero whenever you change wavelengths, switch cell types, or if the instrument has been idle for more than 30 minutes.

7.5. Measuring Samples

1. Insert the sample cell and close the lid
2. Press **Read** (or **Measure** in scan/time-course modes)
3. Record the result (absorbance, concentration, or %T depending on mode)
4. Remove the cell. Rinse and refill for the next sample, or use a fresh cell.

7.6. Wavelength Scan Mode

1. From the home screen, select **Wavelength Scan**
2. Set the start and end wavelengths and the scan increment (typically 1 nm)
3. Zero the instrument with a blank across the full scan range
4. Insert the sample and press **Start Scan**
5. The spectrum is displayed on screen. Use the cursor to read peak wavelengths and absorbance values.
6. Export the scan data via USB (see Section 6.8)

7.7. User-Defined Programs

The DR3900 allows creation of custom calibration curves for analytes not covered by the factory-stored programs:

1. Select **User Programs** from the home screen, then **New**
2. Set the measurement wavelength, units, and cell type
3. Prepare a series of standards spanning the expected concentration range (minimum 5 points plus a blank)
4. Read each standard and enter the known concentration when prompted
5. The instrument fits a calibration curve (linear, polynomial, or segmented). Review the correlation coefficient (r^2); accept only if $r^2 \geq 0.995$ for linear methods (Harris & Lucy, 2020).
6. Save the program with a descriptive name and program number

7.8. Data Export

1. Connect a USB flash drive to the USB-A port on the front panel
2. From the home screen, select **Data Log**
3. Select the readings to export and press **Send to USB**
4. Data are saved as **.csv** files that can be opened in R or Excel
5. Alternatively, connect the DR3900 to a PC via USB cable and use Hach's LINK2SC or Claros software for direct data transfer

7.9. Shutdown

1. Remove all cells from the sample compartment
2. Wipe the cell holder with a dry lint-free tissue to remove any spilled liquid
3. Close the sample compartment lid
4. Turn off the instrument using the rear power switch
5. Cover the instrument with the dust cover if one is available

8. Quality Control and Acceptance Criteria

8.1. Daily Verification

Before each analytical session:

- Run a DI water blank; the reading should be 0.000 ± 0.005 Abs at the working wavelength
- Run a Hach-supplied verification standard (e.g., StablCal formazin standard or NIST-traceable absorbance filter) at the working wavelength. Acceptance: within ± 0.01 Abs of the certified value, or $\pm 5\%$, whichever is larger.

8.2. Lamp Monitoring

The DR3900 tracks lamp hours internally. When a lamp approaches its rated lifetime (typically 2000 hours for deuterium, longer for tungsten), the instrument displays a warning.

- Record lamp hours monthly in the instrument log
- Replace lamps per the Hach service manual or contact Hach technical support
- After lamp replacement, run the full wavelength verification procedure

8.3. Annual Verification

At least once per year (or after any service event):

1. Run a multi-wavelength check using a NIST-traceable holmium oxide filter to verify wavelength accuracy (± 1 nm)
2. Run a multi-point photometric accuracy check using neutral density filters or potassium dichromate standards across the range 0.1–1.5 Abs (Skoog et al., 2018)
3. Record results in the instrument log and retain the verification report

9. Troubleshooting

Symptom	Possible Cause	Action
Self-check fails	Lamp failure or firmware error	Note the error code; contact lab manager
Blank does not zero	Dirty cell, wrong cell type, or stale blank	Clean or replace cell; prepare fresh blank
Erratic readings	Air bubbles in cell, condensation, or dirty optics	Degas sample; wipe cell; clean sample compartment
“Lamp intensity low” warning	Lamp nearing end of life	Schedule lamp replacement
Readings drift over time	Lamp not warmed up; temperature change	Allow 15 min warm-up; avoid drafts near instrument
Absorbance exceeds 2.5	Sample too concentrated	Dilute sample and re-read

10. Waste Disposal

Waste Type	Disposal Method
Rinsate from cells (DI water)	Drain
Chemical samples / reagent waste	Per method SOP (see SOP-L303)
Used lint-free wipes	Standard trash unless contaminated
Spent lamps	Hazardous waste (deuterium lamps contain UV-emitting gas); dispose through EH&S

11. References

- Hach Company. (2024a). *DR3900 benchtop spectrophotometer: Procedures manual*. <https://www.hach.com/dr3900-benchtop-spectrophotometer/product?id=56440352466>
- Hach Company. (2024b). *Water analysis guide* (7th ed.). <https://www.hach.com/wag>
- Harris, D. C., & Lucy, C. A. (2020). *Quantitative chemical analysis* (10th ed.). W. H. Freeman.
- Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of instrumental analysis* (7th ed.). Cengage Learning.

12. Training Record

Before operating the DR3900 independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Read [SOP-L303](#) if performing colorimetric analyses
4. Observe a trained operator perform a complete session: startup, self-check, zeroing, sample measurement, data export, and shutdown
5. Perform a supervised session with acceptable QC results (blank within specification, verification standard within tolerance)
6. Be signed off by the PI or lab manager

13. Appendix A: Instrument Specifications

Parameter	Specification
Wavelength range	320–1100 nm
Wavelength accuracy	± 1 nm
Wavelength resolution	1 nm
Photometric range	-0.5 to 3.5 Abs
Photometric accuracy	± 0.003 Abs at 0.5 Abs (typical)
Light sources	Tungsten (visible), deuterium (UV)
Cell types	10 mm square, 1-inch round, 16 mm round
Stored programs	200+
Data storage	500+ measurements
Connectivity	USB-A, USB-B, Ethernet
Power	100–240 V AC, 50/60 Hz

i Note

Confirm specifications against the serial plate on the unit and the current Hach DR3900 product data sheet. Values above are representative of current production models (Hach Company, 2024a).

14. Appendix B: Relationship to Other SOPs

SOP	Relationship
SOP-L303	Method chemistry, reagent handling, and the analyte
SOP-F208	Method Registry for the DR3900 and DR900
	Field use of the portable DR900 colorimeter
SOP-L212	General spectrophotometer theory and cuvette-based UV-Vis operation Micro-volume UV-Vis (NanoDrop)