



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L303

Hach Colorimetric Analysis Spectrophotometer / Colorimeter

Standard Operating Procedure

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1. Purpose

This procedure describes the general operation, calibration, and quality control framework for colorimetric water analysis using the Hach DR3900 benchtop spectrophotometer and the Hach DR900 portable colorimeter. The SOP is designed as a modular framework: Section 6 covers instrument operation common to all analyses, while the **Method Registry** (Section 7) provides chemistry-specific parameters for each analyte (reagent kit, Hach method number, wavelength, range, reaction time, and interferences). New chemistries can be added to the registry as the lab's needs evolve, without rewriting the core SOP (APHA et al., 2012; Hach Company, 2024a, 2024b).

2. Scope

- **Applies to:** Colorimetric determination of nutrients (phosphorus, nitrate, nitrite, ammonia), metals (iron, manganese, copper, zinc, lead, chromium), and other water quality parameters (silica, sulfate, chloride, hardness) in surface water, groundwater, wastewater, and aqueous extracts using Hach pre-packaged reagent kits and stored programs
- **Does not apply to:** Analyses requiring external standards and user-prepared calibration curves (use the DR3900 in manual wavelength mode with a separate SOP); soil KCl extracts analyzed for $\text{NO}_3^-/\text{NH}_4^+$ via ion chromatography or flow injection (see [SOP-L403](#))
- **Location:** EA Program laboratory, SGM 133 (DR3900); field sites (DR900)

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, review QC data
Lab Manager	Maintain instruments, stock reagent kits, track lot numbers and expiration dates
Trained Personnel	Run analyses per this SOP, record results
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 within the linear range of the instrument (Beer-Lambert law) (Hach Company, 2024a).
- **Beer-Lambert law:** The relationship between absorbance, path length, and concentration: $A = \epsilon cl$, where ϵ is the molar absorptivity, c is concentration, and l is the optical path length; the physical basis for all colorimetric quantification (APHA et al., 2012).
- **Blank (reagent blank):** A sample containing all reagents except the analyte; used to zero the instrument and correct for background absorbance contributed by the reagent matrix (APHA et al., 2012).

- **Continuing calibration verification (CCV):** A known-concentration mid-range standard analyzed at regular intervals during a run to confirm that factory-programmed calibration curves remain accurate; acceptance criterion is $\pm 10\%$ of the stated value (Hach Company, 2024a).
- **Detection limit (MDL):** The minimum analyte concentration reliably distinguished from a blank; method-specific and listed in the Method Registry (§7); the blank must read within the MDL of zero at the start of each session (APHA et al., 2012).
- **DR3900:** The Hach benchtop spectrophotometer; wavelength range 320–1100 nm; uses stored Hach programs or user-defined methods; supports 10 mm square, 1-inch round, and 16 mm round cells (Hach Company, 2024a).
- **DR900:** The Hach portable colorimeter; battery-operated; uses the same stored-program approach as the DR3900; limited to a subset of Hach methods compatible with the 1-inch round cell format (Hach Company, 2024b).
- **Hach method number:** A unique numeric identifier for each Hach reagent-kit and instrument-program combination; used to select the correct stored program on the DR3900 or DR900 and to ensure that the correct reagent is paired with the correct calibration curve (Hach Company, 2024c).
- **Method Registry:** The table in §7 of this SOP listing all validated Hach methods in use in the EA Program laboratory, with analyte, chemistry, program number, reagent catalog number, instrument compatibility, range, sample volume, reaction time, key interferences, and validation status.
- **Powder pillow:** A heat-sealed foil packet containing a pre-weighed, factory-prepared reagent mixture; added directly to the sample to initiate the color-forming reaction; eliminates manual reagent preparation and reduces sources of error (Hach Company, 2024c).
- **RPD (relative percent difference):** $|A - B| / ((A + B) / 2) \times 100$; used to assess duplicate precision; acceptable RPD is less than 20% for concentrations above $5 \times$ the detection limit (APHA et al., 2012).
- **TNT plus vial:** A Hach proprietary pre-dosed reagent vial containing all required reagents sealed in glass; used with the DR3900 for total-nutrient digestion methods (e.g., total phosphorus, total nitrogen) (Hach Company, 2024c).
- **Zeroing:** The process of inserting a reagent blank into the sample cell and pressing Zero to set the baseline absorbance to zero for the selected program; required before each analytical batch (Hach Company, 2024a).

5. Safety and Hazards

 Hazards

- **Chemical:** Hach reagent powder pillows and liquid reagents contain a variety of chemicals depending on the method. Some contain acids (e.g., sulfuric acid in TNT plus vials), heavy metal indicators (e.g., cadmium in older nitrate methods), or oxidizers. Always review the SDS for each reagent kit before first use.
- **Thermal:** COD and total nutrient digestions use the DRB200 reactor block at 150 °C. Use heat-resistant gloves when handling heated vials.
- **Glassware:** Glass vials can break during handling or if heated unevenly. Handle with care; wear safety glasses.

5.1. Required PPE

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

5.2. Emergency Procedures

For reagent splashes to skin or eyes, flush with water for at least 15 minutes. Refer to [SOP-L004 Chemical Spill Response](#) for spill cleanup. For burns from the reactor block, apply cool running water and seek first aid.

6. Equipment

Item	Notes
Hach DR3900 benchtop spectrophotometer	SGM 133 (Hach Company, 2024a)
Hach DR900 portable colorimeter	Field use; see SOP-F208
Hach DRB200 reactor block	For COD and total nutrient digestions (150 °C)
Sample cells (1-inch round, 10 mm square, 16 mm round)	Matched to Hach method; keep clean and scratch-free
Lint-free wipes (Kimwipes)	For cleaning cell exterior before reading
Silicone oil and applicator cloth	For 1-inch round cells to minimize light scatter
Timer / stopwatch	For reaction time control
DI water (squeeze bottle)	For blanks and cell rinsing
Waste container (sealable)	For used reagent vials and solutions

7. Procedure

7.1. Startup and Instrument Check

1. Turn on the DR3900 and allow it to complete its self-check (approximately 30 seconds).

2. Verify the lamp status indicator shows “Ready.”
3. Select the stored program for your analyte by program number (see §7, Method Registry) (Hach Company, 2024a).

7.2. Zeroing

1. Fill a clean sample cell with DI water to the fill line.
2. Wipe the cell exterior with a lint-free cloth. Apply silicone oil for 1-inch round cells.
3. Insert the cell, close the lid, and press **Zero**. The display must read 0.00 (within the method detection limit). If it does not, clean the cell and re-zero.

7.3. Sample Analysis

1. Fill a clean cell to the fill line with the water sample.
2. Add the reagent (powder pillow or TNT vial) per the method instructions.
3. Cap, invert, and mix as directed. Start the timer for the required reaction time.
4. When the reaction time is complete, wipe the cell exterior (apply silicone oil for 1-inch round cells).
5. Insert the cell, close the lid, and press **Read**.
6. Record the result, analyte, program number, sample ID, units, date, and time in the lab notebook.
7. Rinse the cell three times with DI water before the next sample.

7.4. Shutdown

1. Remove all samples and reagents from the instrument compartment.
2. Wipe the cell compartment with a lint-free tissue if any liquid is present.
3. Log the run in the instrument logbook (date, analyst, analytes run, and any instrument issues).
4. Turn off the DR3900 if the session is complete.

8. Method Registry

The tables below list all Hach methods currently validated in the EA Program laboratory. “Active” methods have been verified with known standards; “TBD” methods are pending validation. Add new methods to the registry per §7.4 before routine use.

8.1. Nutrients

Ana-lyte	Hach Method	Pro-gram No.	Reagent / Cat. No.	Instru-ment	Sample Range Vol.	Reac-tion Time	Key Inter-ferences	Sta-tus
Re-ac-tive P (ortho-P)	PhosVer 3 (Ascorbic Acid)	490	2106069 (pillows)	DR3900, DR900	0.02–2.50 mL mg/L PO ₄ ⁻ P	2 min	Silica above 200 mg/L; arsenate	Ac-tive
To-tal Phos-pho-rus	Acid persul-fate diges-tion + PhosVer 3	536	TNT843	DR3900 + DRB200	0.06–3.50 mL mg/L PO ₄ ⁻ P	30 min dig. + 2 min	High turbidity	TBD
Ni-trate (NO ₃ ⁻)	Cad-mium reduc-tion	355	2106169 (pillows)	DR3900, DR900	0.1–30.0 mL mg/L NO ₃ ⁻ N	5 min	NO ₂ ⁻ above 2 mg/L; Cl ⁻ above 100 mg/L	Ac-tive
Ni-trate (NO ₃ ⁻) HR	Chro-motropic acid	410	TNT835/TNT836	DR3900	0.2–30.0 mL mg/L NO ₃ ⁻ N	15 min	Cl ⁻ above 500 mg/L	TBD
Ni-trite (NO ₂ ⁻)	Diazo-tiza-tion	371	2107169 (pillows)	DR3900, DR900	0.002–0.300 mL mg/L NO ₂ ⁻ N	20 min	Strong oxidizers	TBD
Am-mo-nia LR	Salicy-late	385	2668000 (pillows)	DR3900, DR900	0.01–0.50 mL mg/L NH ₃ ⁻ N	20 min	Ca above 1000 mg/L CaCO ₃	Ac-tive
Am-mo-nia HR	Salicy-late HR	343	TNT832	DR3900	0.4–50.0 mL mg/L NH ₃ ⁻ N	15 min	Same as LR	TBD
To-tal Ni-tro-gen	Persul-fate dig. + chro-motropic acid	350	TNT826/TNT827	DR3900 + DRB200	1–25 mL mg/L N	30 min dig. + 15 min	–	TBD

8.2. Metals

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Sample Range Vol.	Reaction Time	Key Interferences	Status
Iron (total)	FerroVer	265	2105669 (pillows)	DR3900, DR900	0.02–3.100 mL mg/L Fe	3 min	Cu above 5 mg/L	Active
Iron (Fe ²⁺)	1,10-Phenanthroline	255	2105769 (pillows)	DR3900, DR900	0.02–3.100 mL mg/L Fe	3 min	Strong oxidizers	TBD
Manganese	PAN	290	Check specific kit	DR3900	0.006–0.700 mL mg/L Mn	2 min	Fe above 5 mg/L	TBD
Copper	Bicinchoninate	135	2105969 (pillows)	DR3900, DR900	0.04–5.100 mL mg/L Cu	2 min	Fe above 10 mg/L	TBD
Zinc	Zincon	780	Check specific kit	DR3900	0.02–3.100 mL mg/L Zn	Immediate	Cu, Pb, Co above 5 mg/L	TBD
Lead	Lead-Trak (dithionite zone)	277	2877200 (AccuVac)	DR3900	5–150 25 mL µg/L Pb	1 min	Cu, Hg, Bi, Sn	TBD
Chromium (Cr ⁶⁺)	DPC	090	Check specific kit	DR3900, DR900	0.010–0.700 mL mg/L Cr	5 min	Fe above 1 mg/L	TBD

8.3. Other Parameters

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Sample Range Vol.	Reaction Time	Key Interferences	Status
Hardness (total)	Cal-MagVer 3	105	2429800 (pillows)	DR3900, DR900	10–4000 mL mg/L as CaCO ₃	Immediate	Mn above 4 mg/L	TBD
Chloride	Mercuric thiocyanate	070	2268500 (pillows)	DR3900, DR900	0.1–25.10 mL mg/L Cl ⁻	Immediate	High turbidity	TBD
Silica	Silicomolybdate	656	2429600 (pillows)	DR3900	1–100 10 mL mg/L SiO ₂	10 min	PO ₄ ; sulfide	TBD
Sulfate	SulfateVer 4	680	2106769 (pillows)	DR3900, DR900	2–70 10 mL mg/L SO ₄	5 min	Silica	TBD

Analyte	Hach Method	Program No.	Reagent / Cat. No.	Instrument	Sample Range Vol.	Reaction Time	Key Interferences	Status
COD	Dichromate reactor	430	TNT821/822/823	DR3900 + DRB200	3–1500 mg/L	2 hr dig.	Cl ⁻ above 2000 mg/L	<i>TBD</i>

8.4. Adding a New Method to the Registry

1. Obtain the Hach procedure document for the method (from the kit box or [hach.com](https://www.hach.com)).
2. Review the SDS for all reagents in the kit.
3. Fill in a new row in the appropriate table above with all required fields.
4. Run the method on at least 3 known standard concentrations spanning the method range to verify that the instrument's stored program produces results within $\pm 10\%$ of the standard value.
5. Run DI water blanks to confirm zero reading.
6. Change the status from *TBD* to **Active** once validation is complete.
7. Record the validation data, date, and analyst in the QC log.

9. Quality Control and Acceptance Criteria

9.1. Every Analytical Session

- **Blank:** Run a DI water blank at the start of each session; must read within the method MDL of zero (APHA et al., 2012).
- **Standard check:** Run one mid-range Hach standard at the start and end of each session; acceptance: $\pm 10\%$ of the stated value (Hach Company, 2024a).
- **Duplicates:** Analyze at least 10% of samples in duplicate; acceptable RPD less than 20% above $5 \times$ the detection limit.

9.2. Calibration Verification

The DR3900 and DR900 use factory-programmed calibration curves that do not require user calibration under normal conditions (Hach Company, 2024a, 2024b). However:

- Run a multi-point verification using Hach-supplied standards at least annually or when the instrument is serviced.
- If a standard check deviates by more than 10%, clean the cell, re-zero, and re-read. If the problem persists, contact Hach technical support.

10. Waste Disposal

Waste Type	Disposal Method
Used powder pillow packets and caps	Standard trash (verify SDS; most are non-hazardous)
Cadmium-containing reagent waste	Hazardous waste; EH&S pickup
Reactor block digestion waste (COD)	Collect in labeled acid waste container
Used sample cells (glass)	Rinse thoroughly; reuse or recycle
Rinse water (DI)	Drain

11. References

- Hach Company (2024a)
- Hach Company (2024b)
- Hach Company (2024c)
- APHA et al. (2012)

APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.

Hach Company. (2024a). *DR3900 benchtop spectrophotometer: Procedures manual*. <https://www.hach.com/dr3900-benchtop-spectrophotometer/product?id=56440352466>

Hach Company. (2024b). *DR900 portable colorimeter: Procedures manual*. <https://www.hach.com/dr900-portable-colorimeter/product?id=56440352476>

Hach Company. (2024c). *Water analysis guide* (7th ed.). <https://www.hach.com/wag>

12. Related SOPs

SOP	Title	Relationship
F208	Portable Colorimeter (Field)	Field use of the DR900
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Reagent waste disposal
L215	Hach DR3900 Instrument	Instrument startup and shutdown

13. Training Record

Before running analyses on the DR3900 or DR900 independently, personnel must:

1. Read this SOP and [SOP-L215](#) in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the SDS for all reagent kits to be used
4. Observe a trained operator perform startup, blank zeroing, sample analysis, and a standard check for at least two analytes

5. Run a full session (blank, standard check, samples, duplicate) under supervision with acceptable QC results
6. Add at least one new method to the registry and validate it before using it on real samples
7. Be signed off by the lab manager or PI