



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L475

Flash EA Soil Combustion for IRMS Analysis

Standard Operating Procedure

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1. Purpose

This SOP describes the complete workflow for analyzing soil and sediment samples for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and elemental C and N content using the Thermo Scientific Flash IRMS Elemental Analyzer (EA) coupled to the Delta V Isotope Ratio Mass Spectrometer (IRMS) through the ConFlo IV continuous-flow interface (Thermo Fisher Scientific, 2008b, 2010a). It covers sample preparation, instrument warm-up, sequence creation, running the analysis, shutdown, and data reduction.

For general instrument operation, gas checks, and pre-analysis tests, see [SOP-L208](#). For the full data correction workflow (blank, linearity, scale normalization, and drift correction), see [SOP-L414](#).

2. Scope

- **Applies to:** Soil, sediment, and plant tissue samples analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and elemental concentrations (%C, %N) by combustion EA-IRMS in NC (dual reactor) mode
- **Does not apply to:** Sulfur isotope analysis (NCS mode), GasBench carbonate analysis (see [SOP-L415](#)), TC/EA hydrogen and oxygen isotope analysis, or liquid samples
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Environmental Isotope Lab, SGM Rm 135

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, ensure training compliance
Lab Manager	Prepare and verify instrument methods; maintain reactors
Super-User	Supervise trainees; perform minor maintenance
Certified User	Prepare samples, build sequences, run analyses
Student Researcher	Sample weighing and data reduction (no unsupervised runs)

4. Definitions

- **Blank correction:** Subtraction of the isotopic and elemental contribution of an empty tin capsule from each sample signal; necessary because capsule tin contributes a small amount of C and N (Paul et al., 2007; Thermo Fisher Scientific, 2010a).
- **Certified user:** A student, staff, or faculty member qualified to prepare samples and run the IRMS without supervision, as designated by the lab PI.
- **ConFlo IV:** Interface hub connecting the Flash EA and other peripherals to the Delta V IRMS; handles reference-gas delivery and peak switching (Thermo Fisher Scientific, 2008a).
- **$\delta^{13}\text{C}$:** Stable carbon isotope ratio expressed in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{13}\text{C}/{}^{12}\text{C}$ (Coplen, 2011).
- **$\delta^{15}\text{N}$:** Stable nitrogen isotope ratio expressed in per mil (‰) relative to atmospheric N_2 (AIR): $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ where $R = {}^{15}\text{N}/{}^{14}\text{N}$ (Coplen, 2011).

- **Drift correction:** Adjustment for systematic temporal change in measured δ values during a sequence, estimated from bracketing reference materials (Paul et al., 2007).
- **Flash EA:** The Thermo Scientific Flash IRMS Elemental Analyzer peripheral; combusts samples at 1020 °C in an O₂ pulse and separates N₂ and CO₂ chromatographically before delivery to the IRMS via the ConFlo IV (Thermo Fisher Scientific, 2010a).
- **Isodat:** Thermo Fisher software for analytical sequence design, instrument control, and raw isotope data export (Thermo Fisher Scientific, 2010b).
- **Linearity correction:** Adjustment applied to account for the dependence of measured δ values on peak amplitude at the detector (Paul et al., 2007).
- **Reference material (RM):** A substance with certified or consensus δ values used to anchor measured values to the VPDB or AIR scale; at least two RMs spanning the expected sample range are required for two-point scale normalization (Coplen, 2011; Werner & Brand, 2001).
- **Scale normalization:** Two-point (or multi-point) correction that maps measured δ values to the international isotope-ratio scale using certified reference materials (Coplen, 2011; Paul et al., 2007).
- **Super-user:** A staff or faculty member qualified to run the IRMS and perform minor maintenance (gas replacement, reactor exchange, etc.).
- **Tin capsule:** 5 × 9 mm pressed-tin container used to encapsulate solid samples; tin ignites in the O₂ pulse, raising combustion temperature briefly to ensure complete combustion.
- **Zero-enrichment test:** Reference gas on/off pulses used to verify that the IRMS measures consistent δ values before analysis; performed during instrument warm-up.

5. Safety and Hazards

Hazards

- **Pressurized gases:** He, O₂, N₂, CO₂. Verify cylinder pressures before each session. He tank pressure must be above 500 psi, regulated to 50 psi.
- **Hot surfaces:** The Flash EA combustion reactor operates above 1000 °C. Use caution when handling ash fingers or reactor tubes.
- **Sharps:** Syringes, tweezers, and capsule-crimping tools can cause puncture or cut injuries.
- **Chemical:** Chromium oxide, silvered cobaltous/cobaltic oxide, and reduced copper used in the reactor packing are irritants; follow standard laboratory handling precautions.

User Responsibilities

The lab manager verifies that methods are working properly, but **Certified Users** are responsible for preparing their own samples, creating run sequences, overseeing runs, and conducting data reduction.

5.1. Required PPE

- ☐ Safety glasses

- ☐ Laboratory coat
- ☐ Nitrile gloves (especially when handling reactor packing materials)

5.2. Emergency Procedures

Refer to [SOP-L208](#) for instrument-specific emergency procedures and [SOP-L002](#) for general lab safety.

6. Required Materials

6.1. Consumables and Reagents (NC Dual Reactor)

Item	ThermoFisher P/N	EA Consumables P/N
Quartz reactor (set of 2)	468 20070	C1069
Ash finger (quartz insert)	N/A	C1098
Silvered cobaltous/cobaltic oxide	338 24500	B1120
Chromium oxide	338 22900	B1099
Reduced copper wires	338 35300	B1016
Quartz wool	338 22200	—
Magnesium perchlorate (Anhydron)	281 13102	—

6.2. Capsules and Weighing Supplies

- Tin capsules (appropriate size for your sample mass)
- Tungsten oxide (WO_3) – approximately 2 parts per sample for combustion aid (if required by method)
- Aluminum crucibles (for oven-drying soils)
- Weighing paper, cell wells, tweezers, spatulas
- Kimwipes, gloves

6.3. Standard Reference Materials

Prepare calibrated internal standards for every batch. Confirm which standards you need **before** approaching the lab. Standards on hand (certified values):

Reference Standard	$\delta^{13}\text{C}$ (VPDB)	$\delta^{15}\text{N}$ (AIR)
LOCSS	−26.66	7.30
HOCSS	−26.27	4.42
Urea	−43.26	−0.56
USGS24	−16.05	—
USGS40	−26.39	−4.52
USGS41a	36.55	47.6

(Coplen, 2011; Werner & Brand, 2001)

! Important

Lab standards are shared resources. Do not use them without approval from a super-user, the lab technician, or the PI. You are expected to replace what you use.

7. Sample Preparation

7.1. Drying and Grinding

1. Weigh soil or sediment samples into aluminum crucibles.
2. Oven-dry at 105 °C until constant mass.
3. Grind dried samples to a fine, homogeneous powder.

7.2. Elemental Pre-screening

Submit a subset of samples to elemental analysis (EA) to determine %C and %N before committing the full batch. This determines the correct sample mass to target the optimal signal range.

7.3. Sample Weighing

The target sample mass depends on %C and %N concentrations (Thermo Fisher Scientific, 2010a):

Typical %C	Target Sample Mass	Notes
> 5% C	3–5 mg	Dilute if signal is too high
1–5% C	7–15 mg	Standard range
< 1% C	15–25 mg	May require longer run times

1. Fold a tin capsule using tweezers (clean hands; no skin oils).
2. Weigh the target sample mass into the capsule.
3. Add WO₃ if required for combustion aids.
4. Crimp the capsule tightly so no sample can escape during handling.
5. Place crimped capsules in sequence order in a cell-well plate.
6. Record the exact mass of each sample in the data reduction spreadsheet.

💡 Tip: Clean Tweezers and Capsules

Oils and residue from fingers contaminate tin capsules, introducing C and N blanks. Handle capsules only with clean, dry tweezers (Thermo Fisher Scientific, 2010a).

8. Instrument Warm-Up and Pre-Analysis Checks

See [SOP-L208](#) for the complete warm-up procedure, gas pressure verification, zero-enrichment test, and linearity check. The Flash EA should be at operating temperature and passing all pre-analysis tests before loading samples.

9. Sequence Design

1. Open Isodat on the acquisition computer (Thermo Fisher Scientific, 2010b).
2. Create a new sequence with the following elements in order:
 - **Empty capsule blanks** (4–6): establish blank baseline
 - **Reference materials** (2–3 each of two or more certified RMs): for scale normalization (Coplen, 2011)
 - **Conditioning samples** (2–3): first samples in the run; typically sacrificial or low-priority
 - **Unknown samples** (bracketed by RMs every 10–12 unknowns)
 - **Closing reference materials** (2–3 of each RM): for drift correction (Paul et al., 2007)
3. Enter sample names, masses, and expected %C/%N for each row.
4. Assign the correct Isodat method to the sequence.
5. Save the sequence file before starting.

10. Running the Analysis

1. Verify carrier gas pressure (He > 500 psi, regulated to 50 psi).
2. Load capsules into the autosampler carousel in sequence order.
3. Press **Start Sequence** in Isodat.
4. Monitor the first 3–4 samples to confirm:
 - N₂ and CO₂ peaks are within the expected mass range
 - Peak shapes are symmetric and well-separated
 - Reference material δ values are within $\pm 0.3\%$ of expected values (Werner & Brand, 2001)
5. If values are drifting, stop the run, identify the cause, and restart after correction.

11. Shutdown

1. After the final sample, allow the sequence to complete fully.
2. Follow the shutdown procedure in [SOP-L208](#).
3. Export the raw data file from Isodat and save to the project directory.

12. Data Reduction

Data reduction follows the protocol in [SOP-L414](#), which covers:

1. Blank correction (empty tin capsule contributions) (Paul et al., 2007)
2. Linearity correction (δ vs. peak area regression)
3. Two-point scale normalization to the VPDB / AIR scale using certified RMs (Coplen, 2011)
4. Drift correction (linear regression through bracketing RMs)
5. Uncertainty estimation and quality flags

13. Definitions of Certification Levels

Level	Capabilities
Lab PI	Unrestricted access; certify super-users
Super-User	Independent operation; certify certified users; replace He, O ₂ cylinders; change reactors
Certified User	All steps from sample prep through data reduction; no unsupervised maintenance
Student Researcher	Sample prep and data reduction only, under supervision

14. References

- Thermo Fisher Scientific (2010a)
- Thermo Fisher Scientific (2008b)
- Thermo Fisher Scientific (2008a)
- Thermo Fisher Scientific (2010b)
- Werner & Brand (2001)
- Coplen (2011)
- Paul et al. (2007)

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25(17), 2538–2560. <https://doi.org/10.1002/rcm.5129>

Paul, D., Skrzypek, G., & F'orizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. *Rapid Communications in Mass Spectrometry*, 21(18), 3006–3014. <https://doi.org/10.1002/rcm.3185>

Thermo Fisher Scientific. (2008a). *ConFlo IV universal interface: Operating manual*.

Thermo Fisher Scientific. (2008b). *Delta V isotope ratio MS: Operating manual*.

Thermo Fisher Scientific. (2010a). *Flash IRMS EA: Operating manual*.

Thermo Fisher Scientific. (2010b). *Isodat 3.0 software: User guide*.

Werner, R. A., & Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, 15(7), 501–519. <https://doi.org/10.1002/rcm.258>

15. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	General safety framework
L208	Delta V IRMS General Instrument	Instrument startup and QC
L414	Soil CN by Flash EA-IRMS	Full data correction workflow
L415	Carbonate GasBench IRMS	Complementary IRMS application

16. Training Record

Before running analyses independently, personnel must:

1. Read this SOP and [SOP-L208](#) in full
2. Be assigned a certification level by the Lab PI
3. Observe a Super-User or Certified User run a complete sequence from sample loading through data reduction
4. Run a supervised batch of at least 10 unknown samples with RM checks, achieving results within $\pm 0.3\%$ of expected values for both C and N
5. Be signed off by the Lab PI or designated Super-User