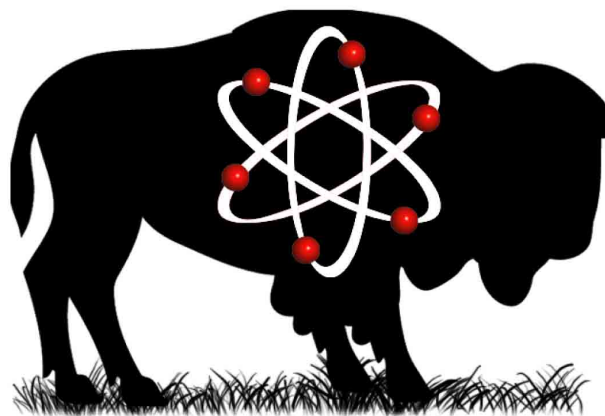


**University of Wyoming Stable Isotope Facility**  
**Test Method for NEON**  
***010 EA-IRMS Organic d15N, d13C, C% & N%***  
**June 2017**

*Adapted from the UWSIF User Manual, 2014*



UNIVERSITY OF WYOMING  

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**Stable isotope Facility**

## TABLE OF CONTENTS

A	Overview of UWSIF .....	3
A.1	General description of the UWSIF .....	3
A.2	Oversight and management.....	3
A.3	QA/QC guidelines.....	4
A.4	Sample tracking guidelines .....	5
A.5	Training for employees .....	6
B	Sample Preparation .....	7
B.1	Soil carbonate removal by acidification (as needed for dryland sites) .....	7
B.2	Grinding .....	7
B.3	Sample weighing - All samples and reference materials are prepared in the same manner.....	8
B.3.1	Elemental analyzer - use tin capsules only .....	8
C	Standard Operating Procedure for the Costech® Elemental Combustion System coupled with the Thermo® Finnigan Delta Plus XP Mass Spectrometer .....	9
C.1	Sample Loading and Preparation .....	9
C.2	Current Instrument State .....	9
C.3	Recent Sample Verification and Data Processing using IsoDat Software.....	10
C.4	Setting up a Sample Table using IsoDat Software.....	12
C.5	Starting the Analysis using IsoDat Software .....	12
C.6	Retrieving Data using IsoDat Software .....	13
C.7	Changing EA Columns .....	13
C.7.1	Column change .....	13
C.7.2	Performing a peak jump calibration.....	14

## **A Overview of UWSIF**

### **A.1 General description of the UWSIF**

The Stable Isotope Facility is a core research facility at the University of Wyoming. We provide quality isotopic analyses for the research community at UW and other universities and labs nation-wide. We are engaged in development of novel analytical techniques, advice researchers on sampling and analysis and serve as a hands-on teaching facility for UW students, post-doctoral associates and faculty. The UWSIF is dedicated to ecological and environmental applications of light stable isotopes (H, O, C and N) and is fully equipped and staffed for a broad suite of applications. We play a prominent role in helping on-campus researchers address environmental management and natural resource issues in Wyoming.

The facility is equipped with two Finnigan Delta+XP continuous flow inlet Stable Isotope Ratio Mass Spectrometers (IRMS), a Micromass Optima Dual Inlet IRMS, and a Micromass IsoPrime continuous flow IRMS. All instruments are configured for the analysis of H, C, N, and O. These mass spectrometers are connected to eight different peripheral devices for automated sample processing, helping to meet the growing demands of researchers and students on campus. The SIF is acquiring an isotope laser spectrometer with auto injector (Los Gatos Inc.) for laboratory analysis of the stable isotopic composition of liquid waters. The UWSIF also has vacuum extraction lines for off-line preparation and purification of sample gases, sample storage freezers, ovens, a fume hood, centrifuges, grinding mills, and other miscellaneous sample preparation equipment.

### **A.2 Oversight and management**

The UWSIF is administered by three permanent faculty and staff members. The Director (faculty member with 25% service appointment) sets the vision and broad objectives for the lab and provides guidance, leadership and oversight for the operation and long-term growth of the facility. The Associate Director/Manager of the UWSIF (PhD. Level AP Research Scientist with 65% service appointment) helps to shape the long-term objectives for the facility, provides isotope consultation to researchers across campus, prepares annual reports for UWSIF, prepares job reports for clients, in-charge of hiring and providing technical guidance to all technicians working in the facility and conducts tours of the facility for visiting Faculty candidates and researchers/administrators on campus. A full time, permanent technician (staff scientist with 100% appointment to the UWSIF) is responsible for the technical maintenance of the instrumentation, sample analyses and routine day to day operations of the facility.

Broader oversight of the UWSIF is provided by a steering committee of 6 faculty members from different departments on campus (i.e., Botany, Zoology and Physiology, Geology and Geophysics, Archaeology and Anthropology and Renewable Resources). The role of the steering committee is to help provide guidance on overall strategic direction of the UWSIF and to provide feedback and oversight on technical challenges, policy issues and financial management of the lab. The steering committee meets with the UWSIF Director and Associate Director/Manager once in both Spring and Fall semesters. These meetings also provide the opportunity to discuss future strategies to be adopted by UWSIF to keep pace with the upcoming technologies. The committee composition will reflect balance among participating academic units across campus and allow other new faculty to participate in the evolution of the facility. A major goal is to have participation from faculty with diverse technical requirements and disciplinary backgrounds. Membership will be on a **two-year rotation**, but with each member having the opportunity to serve on a continuing basis (negotiated with Director) as necessary to meet goals for the facility.

### **A.3 QA/QC guidelines**

To provide accurate isotopic data to internal and external users we take the following steps to assure the long-term consistency and international comparability of the isotope data produced from the facility:

1. To keep a check on linearity of the mass spectrometers a reference gas linearity test is performed by spanning the 1000mv-8000mv signal range once every 6 months or at the time of any instrument service (i.e. cleaning of the source, replacement of filament, etc.) whichever is earlier.
2. A set of internal laboratory working standards have been developed and calibrated against IAEA, NIST and CMDL primary standards. Primary calibration on these working standards is conducted every 1-2 years, when a new standard is added, or at the time of any instrument service, whichever is earlier.
3. About 20% of all samples analyzed in the lab are working standards, including standards for data normalization, and check standards. The purpose of the two types of standards are:
  - A) **Normalization Standards:** The normalization standards are calibrated with primary standards using a three-point calibration curve. These working standards are then used in routine analyses distributed at the beginning, middle and end of each run. Besides providing the normalization curve they also help to keep a check on issues like machine drift, non-linearity related to sample size, and column degradation.
  - B) **Quality Check Standards:** These standards provide a check on the two-point normalization curve produced by the normalization standards. One

sample is placed in the beginning and end of each run. These standards have similar matrix to the unknown samples being analyzed for the clients. They also provide a means to check for consistency between runs.

4. Raw and corrected data are provided to the clients along with analysis data for all standards used in each analysis run. That way client can verify that proper QA/QC procedures were followed.
5. All analysis data are archived electronically using two separate master back-up hard drive systems within the UWSIF for long-term storage and future retrieval.
6. We maintain long-term record of our normalization and check standards which serves as an “**Early Warning Signal**” to detect any minor/major isotopic or sensitivity shifts in our instruments.
7. When possible we participate in inter-lab comparison exercises to evaluate the robustness of our QA/QC guidelines.

#### **A.4 Sample tracking guidelines**

To keep track of samples from the moment they arrive at the laboratory to the time results are reported, the analysis jobs are managed using tracking paperwork and the QuickBooks database system. The tracking paperwork and database records list the specific analyses to be performed, the cost for the service, the number and the type of samples for analysis, and pertinent contact and billing information for the client. Progress during analysis for each job is tracked continuously, and all jobs in progress are managed and organized daily and weekly by the lab manager and lab technicians. The sample tracking system works as follows:

1. New samples are submitted to the lab via a Routing Sheet (Shipping Manifest in the case of NEON). Each routing sheet is assigned a new job number e.g. 2007-0031, 0032 etc.
2. The samples retain their unique id assigned by the PI when they are entered in the sample sequence table of the Finnigan software.
3. Each sample is also given a unique UWSIF number generated by an excel macro when the job is checked in. This number includes the routing sheet and a unique number pertaining to the sample e.g. 20070031.001
4. For sample traceability the File name assigned to each run is same as the job number. If one job has several runs they are named sequentially e.g. 2007-0031 run 1 will be followed by 2007-0031 run 2 and so on.
5. The results are sorted in different folders according to analysis type and PI’s last name.
6. When analysis is done, a complete report is sent to the PI along with an invoice containing details of Job number, analysis type and final sample number.

## **A.5 Training for employees**

### **Lab Safety**

All employees are required to take a lab safety course before performing any laboratory work. The laboratory course is offered by the University of Wyoming Environmental Health and Safety. Employees take a refresher course every three years. The courses taken are as follows:

- 1.) **Safety Orientation** - Workplace safety responsibilities of UW and employees, common hazards for all employees, accident prevention and reporting, role of EHS.
- 2.) **Laboratory Safety - Chemical Hygiene (OSHA Lab Safety)**-Covers chemical safety in the laboratory; general chemical safety; responsibilities; and liabilities.
- 3.) **Hazardous Waste Generator**- Regulations for disposal of hazardous waste, UW program and procedures for disposing of hazardous waste.

### **Instrumentation and UWSIF training**

Each employee is trained in the facility on the instruments pertaining to their experience and skill. The employee is given a walk through from an experienced person for each instrument or SOP. They are shown how to do the tasks and then supervised throughout the training process. Once it is deemed they are proficient at the task, they are allowed to work on their own. A training checklist is filled out for each employee and kept on record.

### **Off-site training**

Employees are occasionally sent to training courses off-site at manufacturer's training facilities. This is done when a new instrument is purchased and specialized training is needed in order to understand how to run new instrumentation.

### **Other Training**

Other training is deemed necessary by the facility director or faculty director based on the needs of the facility.

## **B Sample Preparation**

### ***B.1 Soil carbonate removal by acidification (as needed for dryland sites)***

Note: The reaction of acid (phosphoric in this case) with carbonates in the soil releases CO<sub>2</sub> thereby removing any inorganic C in the sample that may confound isotopic analysis of organic C.

- 1) Weigh out 100mg of finely ground soil into clearly labeled 2 ml eppendorf tubes.
- 2) Cautiously add 50ml of 3N phosphoric acid to eppendorf tube. Sample will bubble as carbonate is converted to CO<sub>2</sub>, do not close the lid.
- 3) Next close lid when bubbling stops and vortex each tube. (make sure to open lid after being vortexed)
- 4) place tubes in the centrifuge and spin at ~2800 rpm for 15min.
- 5) Carefully decant the supernatant into a waste beaker. To avoid losing any soil particles while pouring off liquid, leave a little liquid in the tube. Successive rinses will remove any residual acid.
- 6) Repeat steps 2-6 two more times or until pH is 0 (each time testing the pH)
- 7) Repeat steps 2-6 using DI H<sub>2</sub>O instead of acid. At this point all inorganic C is gone, and soil must now be rinsed to remove any acid.
- 8) Rinse two more times with DI H<sub>2</sub>O. Tubes DO NOT need to sit overnight (step 4 may be omitted to save time).
- 9) After final rinse with DI H<sub>2</sub>O, samples should be placed in drying oven at 100° C for 24 hours.
- 10) Samples are now ready to be weighed out for isotopic analysis.

### ***B.2 Grinding***

There are two different types of containers that can be used to grind samples with the Retsch ball mill depending on the sample amount needing to be ground. For large samples use the stainless steel containers where you can grind two samples at a time. For small samples the eppendorf grinding jars can be used.

- 1) Load samples into desired container
- 2) Add grinding balls to container
- 3) For the 25mL jar use 2- 5(10mm) ball charge pieces per jar
- 4) For the eppendorf tubes use 1-2(5mm) ball charge pieces per tube
- 5) Place grinding jars into the grinder and tighten down the primary and the secondary clamp

- 6) Set the desired frequency and time on the top of the ball mill by turning the dials
- 7) Check the consistency of the samples and make sure they are homogenous
- 8) Remove the charge balls using a magnet
- 9) Clean containers and charge balls with ethanol and place in the oven to dry

**B.3 Sample weighing - All samples and reference materials are prepared in the same manner.**

**B.3.1 Elemental analyzer - use tin capsules only**

- 1) Place a tin capsule on the microbalance close the door and tare the balance
- 2) Remove the capsule and place the desired amount of sample into the capsule. Test to make sure you have the weight in the correct range for the sample type.
- 3) If the weight is incorrect remove or add material until it is in range.
- 4) Once the desired weight is accomplished remove the capsule and crimp it into a round shape without any protrusions.
- 5) Place the capsule back on the balance and record its final weight
- 6) Place the capsule in the designated well in the well plate



## **C Standard Operating Procedure for the Costech® Elemental Combustion System coupled with the Thermo® Finnigan Delta Plus XP Mass Spectrometer**

### ***C.1 Sample Loading and Preparation***

- Samples must be in tin capsules.
- The samples must not be too large for the carousel and they must not be flat.
- The proper weight of the sample substance should yield sufficient amplitudes between approximately 2000 and 3000 mV.
- Release the pressure from the sample carousel. There is a purge line inlet located on the top of the autosampler lid in the center.
- After the pressure has been released open the lid by turning the three lid locking bolts. Due to the sealed nature of the lid always tighten and loosen lid locking bolts in an even random nature (As you would with lug nuts on a car tire).
- You may need to change the carousel to 50 or 100 depending on the size of the sample. Be sure to also change the carousel size on the EA Panel.
- Line up the carousel if necessary.
- Load samples. The first sample should be located to the right of the combustion chamber.
- Close the autosampler lid.
- Leave the purge line inlet open for 10 minutes to allow for the evacuation of any remaining atmosphere within the sample carousel. Double check that the helium dilution is on.
- After the 10 minute purge close the purge line inlet and perform a helium leak check.

### ***C.2 Current Instrument State***

- The mass spectrometer (MS, also referred to as the Thermo Finnigan Delta Plus XP Mass Spectrometer) and the elemental analyzer (EA, also referred to as the Costech Elemental Combustion System) communicate with the Isodat and EAS program as long as the EA is in remote mode (noted on the EA as REM).
- The state of the mass spectrometer is dynamic and if great fluctuations are observed notify a supervisor before any other actions are performed. Normal values are as follows:

- High Voltage [KV] 2.950 (varies)
  - Magnet [Steps] (varies)
  - HV [KV] 3.01
  - Box [mA] 0.8
  - Vac [m] 1.0e-006
  - Trap [mA] 0.70
- Check for a background mass of 28 by right clicking on the center bar within the MS panel located on the left side of the computers display screen and clicking on JUMP TO MASS and entering 28. After the instrument changes have taken place right click the center bar again and perform a PEAK CENTER check. If the peak center is erratic wait for stabilization then retest.
  - Perform the necessary on offs. For example if you are running samples that will only utilize one reference gas then that is the only gas line that will need to be tested. After an on off set has been completed you are able to check the standard deviation of the peaks. Skip the first two injections and highlight the rest of the appropriate column (for example 13C or 15N) and then right click the highlighted area and choose the CALCULATE option. If the standard deviation of the last on off performed is below 0.03 then you are able to move on to the next step. If the value is higher than 0.03, but the standard deviations are decreasing it is possible to start the run. It is important to check the first few standards to make sure the values are reasonable. Normally 3 sets for each type of on off are more than sufficient. It is best not to spend too much time performing on offs.

### ***C.3 Recent Sample Verification and Data Processing using IsoDat Software***

It is necessary to check the results from the previous run to ensure that no problems were encountered.

- Open excel.
- Go to: Open, Results, and select the name the run was saved under.
- You will find a number of generic file folders named “Excel” followed by a numeric number. Check the date of the excel file to ensure that is the appropriate file (the file and therefore the date is automatically generated when the run is complete). It is usually the last excel file at the end.
- Open this folder and then its lone excel file (again with an automatically generated name).

- Move and copy the contents of the excel file to a new excel document by right clicking on the worksheet tab and choosing the “move or copy”. Check the box that says make copy and press “ok”.
- Close the original file.
- Save the new excel file on the desktop under EA Results in the folder containing the clients name with a file name including the project code and the run number if applicable (i.e. 2008-0008 run 1 Client Name).
- Copy the data from sheet 1 to sheet 2
- Name sheet 1 “Original”, sheet 2 “Sorted”. Insert EA MS worksheet and rename run 1 or the appropriate run number.
- Sort the data in “Sorted” sheet by peak number (Select Peak number column, DATA, SORT, PEAK #).
- For 13C and 15N analyses delete the reference peaks 1, 2, 5, and 6 for 13C and 15N analysis. For 13C only delete peaks 1, 2, and 4.
- Move the data from peak 4 (under the columns amp 44, d13c/12c, %) sequentially into the spaces above.
- Delete all of the remaining data from peak 4.
- Delete the Peak Nr column.
- Copy this data to sheet 3(EAMS RUN) and if you are including EAS data do so now (see section 3.4.2). If you are *not* including EAS (%) data you will need to delete the unnecessary cells.
- Sequentially (from the beginning to the end of the run) copy all of the labs standards to the bottom of the run into their appropriate position.
- Check to make sure that the values received are sufficient and that there are no problems with the run. It is important to check the standard deviation of all standards. The standard deviation for the isotope values should be less than 0.1 and for the EAS/percent data, if included, should be below 0.5. If not, you may need to look at the individual values.
- Remove the first three standards from the run.
- If necessary you can perform a q-test on an individual standard that has a value which is far off from the rest.
- It is also important to look for a possible drift, represented by values getting sequentially higher or lower throughout the run.
- If a drift or linearity correction is needed then do appropriate corrections.
- Check that all Amplitudes of standards and samples are above 500. If not, this means that the sample amount is insufficient to produce reliable isotope ratio values and should be reported to the PI.
- Copy the line equation from the normalization graph into the appropriate column (carbon graph into carbon column, nitrogen graph into nitrogen

column.) replace the x in the formula to \* and click on the matching row and column for d 13C/152C for carbon and D 15N/ 14N for nitrogen. Copy and paste that number into remaining cells that need a correction.

- Repeat for the nitrogen correction.
- To check the run, copy the average corrected value for your check standard in the Lab QC Check box matching the appropriate check standard. The difference between the corrected value and the actual value must be within 0.4 for nitrogen and 0.3 for Carbon.
- In the Lab QC check box delete any standard not used in the run.
- Delete standards at the top of the page that are dispersed among the samples.
- Save

#### **C.4 Setting up a Sample Table using IsoDat Software**

- In excel, open the sample sheet folder on the desktop
- Find the clients folder and open the correct sample sheet for the run.
- Open Isodat workspace. If the samples need to be analyzed for carbon and nitrogen open the sequence tab and double click the N2+CO2+He sequence. If only carbon or only nitrogen analysis is needed double click the CO2 only or N2 only sequence.
- Copy and paste, from excel, the sample ID's into the identifier column.
- Copy and paste, from excel, the sample weights in the amount column.
- Double check to ensure that the method column is consistent throughout all samples.
- Check that you have all the samples that were in your sample sheet.
- Check that the method column has the correct method and all cells are filled.
- Highlight the whole sample table by clicking on the box in the upper left hand corner of the sample table and save it, no file name is needed. The saved data will automatically be carried over to Isodat acquisition when the corresponding sequence is opened. Sometimes the sequence must be closed, and then opened again for the new data to register.

#### **C.5 Starting the Analysis using IsoDat Software**

- Perform the steps of sections 3.1-3.3 and 3.5.
- If the current run's sample table is not active make it so by double clicking on the appropriate sequence (example N2+CO2+He).

- After the mass 28 has reached a mV of less than 10 and is stable (wait approx. 10 minutes after changing the mass to 28) highlight the entire sample table and press start. If running the EAS software concurrently start the EAS before Isodat.

## **C.6 Retrieving Data using IsoDat Software**

- To view all data in excel format follow section 3.4.1.
- To view the sample chromatograms open the RESULTS tab under the file browser on the left hand side of the Isodat screen and navigate to the place where your sample's data was saved.

## **C.7 Changing EA Columns**

### **C.7.1 Column change**

- Be sure to wear leather gloves and safety glasses while changing columns.
- Close the mass spec. by turning the valve **carefully** until you see the vacuum pressure in isodat drop to xxe-009. **Do not overtighten.**
- On the EA instrument control panel, select std-by mode button, then enter.
- Change left oven to 500 degrees.
- Once both ovens have reached 500 degrees or below.
- Take out the old columns by unscrewing the bottom and then the top.
- Insert new columns and water trap. Make sure all O rings are still in good shape before reusing. (O rings should be flexible and cracks should *not* be visible.)
- Make sure the metal props, under columns, are secure and put EA in work mode.
- Perform a helium leak check to all seals and openings including carousel. You may have to retighten or replace O rings in order for there not to be leaks. (Be sure to turn off helium before retightening anything. You may need to open the green valve on autosampler for the pressure to release before opening anything again.)
- Once there are no leaks the left oven temperature can be returned to the original temperature (1020°C) and EA should be placed into work mode by pressing work button and enter.

- Once ovens have reached their working temperatures, open the mass spec. by turning the valve **carefully** until you see the vacuum pressure rise to approximately  $1e-006$ .
- Ensure that the nitrogen background is below 10 mV by performing a jump to mass 28 and a peak center.

### C.7.2 Performing a peak jump calibration

- **Peak jump calibration should be performed after a filament has been changed or when the carbon standard deviations are high.**
- Open Instrument Control on the desktop.
- Choose Configuration “conflo solids”.
- Turn to the devices window.
- Open Reference 2 (CO<sub>2</sub>) by clicking on Ref 1 capillary. Thus, the blue capillary symbol elongates and CO<sub>2</sub> enters the system.
- Return to the instrument Control window.
- Select **Scan > Jump Calibration** or alternatively; on the toolbar next to the Instrument Control window press the **Jump** button.
- In the list of available Jump Calibration mark the one for N<sub>2</sub>→CO<sub>2</sub> by clicking is No. (e.g. 001).
- Press **Recalibrate** icon.