

Smart Notes



How do I measure clumped $\Delta 47$ from carbonates using the Thermo Scientific portfolio?

What are clumped isotopes?

Clumped isotopes refer to rare isotopologues in natural materials which are characterized by having heavy isotopes (D, ^{13}C , ^{15}N , ^{18}O , etc.) bonding with or near each other rather than with the light isotopes¹. These rare isotopologues can be used to investigate past climate changes by reconstructing temperatures from diverse archives. Paleoclimate reconstruction is using carbonate clumped isotopes thermometry to tackle temperature dependence of the abundance of ^{13}C - ^{18}O bonds in the carbonate lattice². Paleo-studies are undertaken on samples such as foraminifera, omnipresent fossil species that precipitate calcite shell. These shells are commonly made of calcium carbonate and therefore offer an excellent source for carbonate clumped isotopes analysis. Due to their limited availability in certain samples and extensive labor work, required to manually pick out individual foraminifera, highest sensitivity at full precision is needed for performing clumped isotope analysis on IRMS systems.



How can clumped isotopes be measured?

The analysis of clumped isotopes requires Isotope Ratio Mass Spectrometry.

Thermo Scientific solutions for the analysis of clumped isotopes include:

- Thermo Scientific™ Kiel IV Carbonate Device
- Thermo Scientific™ 253 Plus 10 kV IRMS
- Thermo Scientific™ Isodat Software Suite with Thermo Scientific™ LIDI 2 Software Workflow

The analysis of carbonate clumped isotopes with the Thermo Scientific portfolio is a step-by-step process, from sample weighting to results allowing precise and accurate determination of $\Delta 47$ from small samples.

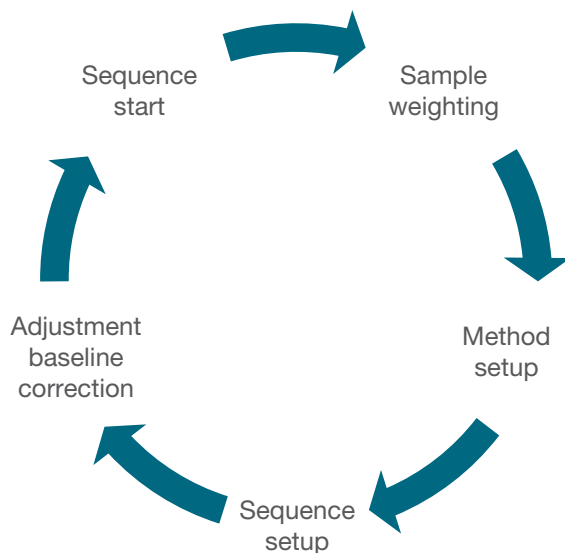


Figure 1. Workflow for clumped isotopes analysis.

The Kiel IV Carbonate Device is an automated sample preparation unit employing acid digestion of carbonates with ~105% H_3PO_4 . The released CO_2 is purified from water using a cryogenic trap and cryo-focused in a 50 μL microvolume before measurement. Optionally, a Peltier-cooled Purification Trap can be installed in the Kiel IV Carbonate Device for removal of potential organic contaminants. For measurement of the generated sample CO_2 , the 253 Plus 10 kV IRMS is used, equipped with a dedicated collector for isotopologues on masses 44 to 49 and monitoring of the pressure baseline.

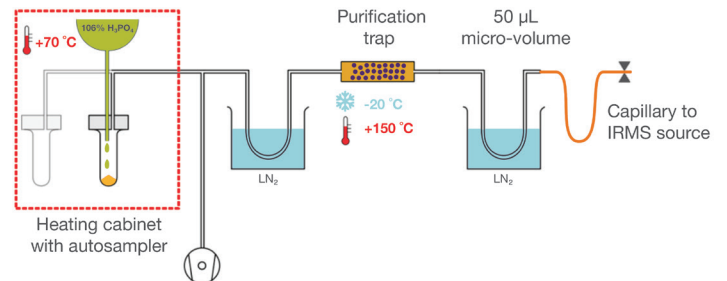


Figure 2. Simplified schematic layout of the Kiel IV Carbonate Device.

Analytical set-up

For accurate and precise measurement of clumped $\Delta 47$ from carbonate minerals, the following prerequisite are required:

- Capillary matching (crimping)
 - Good matching of the sample and reference signal is the key to obtaining accurate and precise data. The mismatch between sample and reference signal upon depletion of the micro volumes should not exceed 50 mV for LIDI
- Determination of “signal up” parameters
 - This refers to the increase in signal when the microvolume is closed off. The “signal up” parameters for Dual Inlet sample and the reference side are determined once after the capillaries are matched
- Preparation of Phosphoric acid
 - Prepare phosphoric acid with a concentration between 104 and 106%
- Preparation of the adsorption trap (optional)
 - Before the first measurement and on a regular basis afterwards, the trapping material must be replaced. The frequency of replacement solely depends on the kind of samples which are measured. A replacement every 1 to 3 months is sufficient for most applications

- Reference refill time
 - Determine the time needed to fill the reference bellows via the automatic refill. The bellows are considered sufficiently filled when a signal of 25000 mV on mass 44 is reached at 25% compression

Measurement workflow

Sample weighting

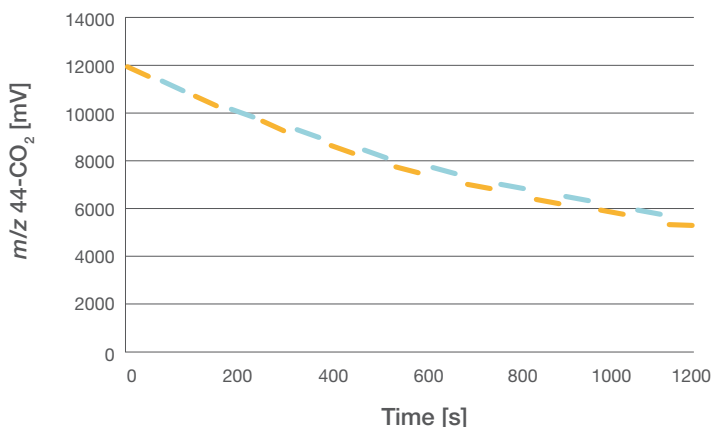
Carbonate samples for clumped isotope measurement must be stored in sealed vials inside a dry cabinet to avoid any impact from moisture.

- Clean all equipment used for weighing (spatulas, tweezers) with analytical grade methanol,
- Weight 80 to 120 μg of sample using a high precision balance into aluminum weighting pans,
- Transfer samples into the Kiel IV Carbonate Device vials and ensure that all carbonate accumulates at the bottom of the vial
- When using the LIDI 2 Software Workflow, sample weights need to be within a narrow range

After weighting, the samples are measured immediately, or stored in the upper section of the Kiel IV Carbonate Device oven at 70 °C, thereby excluding condensation of moisture.

Long Integration Dual Inlet (LIDI)

The Kiel IV Carbonate device is designed for small carbonate sample amounts. To obtain the highest precision with these samples, optimal sample utilization is required. This is achieved by LIDI (Long Integration Dual Inlet), where sample and reference are measured consecutively (Figure 3).



LIDI 2 Software Workflow offers drift correction by standardization of the sample data with two reference data sets (“Bracketing”). When lab temperature fluctuation exceeds 0.1 °C/h, the usage of LIDI 2 Software Workflow is recommended. Bracketing may be omitted, especially when the amount of CaCO_3 differs significantly between the samples.

Setup of the method

Setup a method for LIDI using the parameters in Table 1. Only the parameters different from the default settings are shown. The time for the reference refill and the “signal up” parameters need to be determined as described. Please note that the following parameters are recommendations only and may differ depending on sample type employed.

Table 1.

Instrument	
Interference correction	Enabled for automatic pressure baseline correction
Acquisition mode	LIDI
Bracketing	Enabled for LIDI 2 Software Workflow Disabled for LIDI
Integration time	10 s
Refill time	As determined
Peripherals	
Number of cycles	60 s
Idle time	40 s
Bellow master	Sample
Ref signal up	As determined
Cooling temperature*	-20 °C (recommended)
Baking temperature*	150 °C (recommended)
Baking time*	60 min (recommended)
Cool after baking*	Enabled, if next sequence follows within a day

* only available with optional Purification Trap

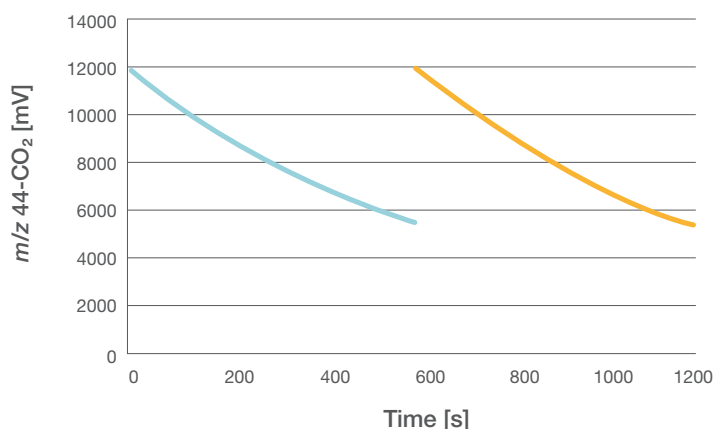


Figure 3. Signal sequence for one conventional Dual Inlet measurement (left) and one LIDI measurement (right). Blue: Sample, Yellow: Reference data.

Cool Trap Bake Trap

Row					Reference Refill	Line	Sample	Weight [mg]	Identifier 1	Identifier 2	Comment	Preparation	Method
1	✓	✓	✓	✓		1	2	82	ETH 1				Kiel IV LIDI.met
2	✓	✓	✓	✓		2	2	88	ETH 3				Kiel IV LIDI.met
3	✓	✓	✓	✓		1	3	89	Sample 1/1				Kiel IV LIDI.met
4	✓	✓	✓	✓		2	3	94	Sample 1/2				Kiel IV LIDI.met
5	✓	✓	✓	✓		1	4	79	ETH 3				Kiel IV LIDI.met
6	✓	✓	✓	✓		2	4	93	ETH 1				Kiel IV LIDI.met
7	✓	✓	✓	✓		1	5	86	Sample 2/1				Kiel IV LIDI.met
8	✓	✓	✓	✓		2	5	80	Sample 2/2				Kiel IV LIDI.met
9	✓	✓	✓	✓		1	6	79	Sample 3/1				Kiel IV LIDI.met
10	✓	✓	✓	✓		1	6	93	Sample 3/2				Kiel IV LIDI.met

Figure 4. Layout of a typical sequence.

Typically, a sequence consists of several standards and samples as shown below. While the sorting of samples and standards inside the sequence is solely dependent on the standardization approach, it is recommended to measure each standard on both sample lines. This facilitates the identification of a contamination affecting one of the sample lines.

If the adsorption trap is used, it should be set to “Cool mode” for every sample. For most samples, baking of the adsorption trap is only performed at the end of the Sequence.

Adjustment of the automatic baseline correction

The clumped-CO₂ collector of the 253 Plus 10 kV IRMS is equipped with a cup dedicated to baseline monitoring. The beam intensity on *m/z*=47 is corrected for by using the baseline signal and k Factor, which is a coefficient describing the proportionality between the baseline on the monitoring cup and on the mass 47 cup (Figure 5). Control of the k factor is recommended on a daily basis.

The k-Factor for mass 47 is determined in Instrument Control using the following procedure:

- Important – before determining the k-Factor, disable the automatic baseline correction. If enabled, Instrument Control shows signals which are already corrected
- Adjust the focussing as for measurement
- Set the instrument to the reference bellow and adjust the beam intensity (*m/z*=44) to 15000 – 20000 mV. It is good practice to use the same intensity each time the k Factor is determined
- Perform a peak shape scan across the whole mass 47 peak plus >20 V on both sides
- Visually determine the pressure baseline on the mass 47 peak as shown in Figure 5. Preferably, use spreadsheet software (e.g., Microsoft™ Excel)

- The relevant baseline values ⁴⁷I and ^{47.5}I are obtained from this pressure baseline and the baseline on the monitoring cup at the position of the peak centre
- The k factor is given by the equation:

$$K = - \frac{47I}{47.5I}$$

Attention: The above equation considers both ⁴⁷I and ^{47.5}I to be negative.

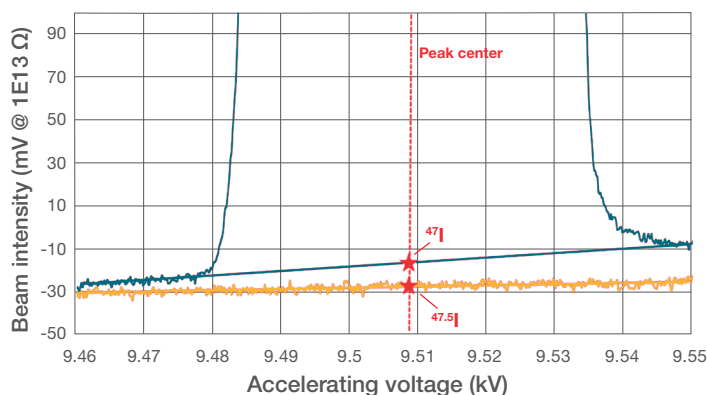
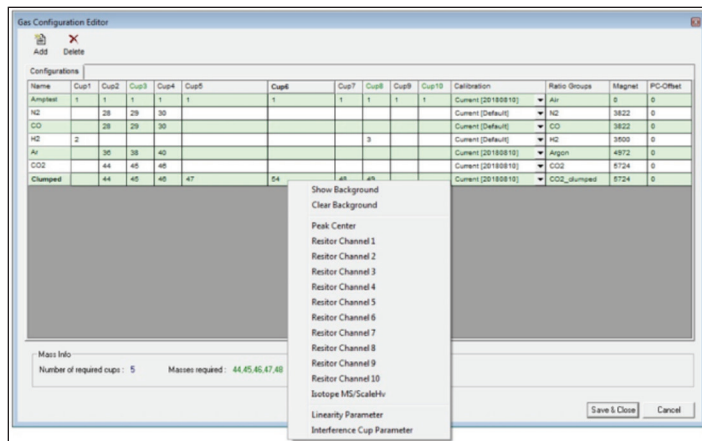


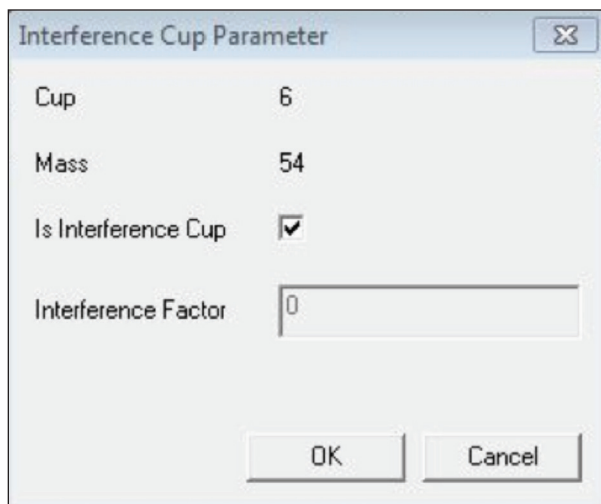
Figure 5. Determination of the k-Factor.

Entering the k-Factor in Isodat Software Suite

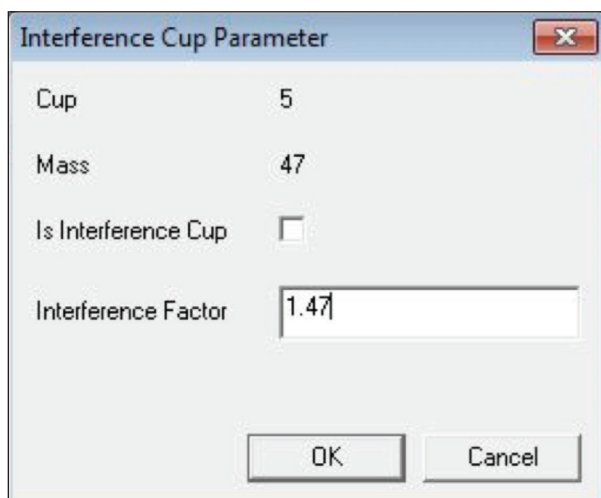
1. Open the Gas Configuration editor
2. Right click on the Cup used for baseline monitoring (here: Cup 6)



3. Select "Interference Cup Parameter"
4. Enable "Is Interference Cup"



5. Right click on the mass 47 cup, select "Interference Cup Parameter"
6. Enter the determined k-Factor (here: 1.47)



7. Confirm with ok. The Gas configuration now shows the equation used for baseline correction

Clumped		44	45	46	47	(+1.47*Cup6)	Interference	48	49		
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Running the sequence

Before starting the sequence, it is recommended to perform some quick cleaning procedures on the Kiel IV Carbonate Device:

- Wipe off any particles from the O-rings which seal off the vials. Occasionally, a small amount of high temperature grease should be put on the O-rings
- Using lint free paper, carefully remove droplets of phosphoric acid which may be present at the tip of the acid capillary or the drop detection wire
- Release 20 to 30 drops of the acid into a pair of separate vials. Discard this acid.

Start the Sequence in Isodat Software Suite. The following steps will now be executed automatically:

- Setting the purification trap to the desired cooling temperature (if available)
- First Reference measurement when LIDI 2 Software Workflow is used
- First sample preparation and measurement
- Further reference measurements, sample preparations and measurements
- Baking of the Purification trap at the end of the sequence

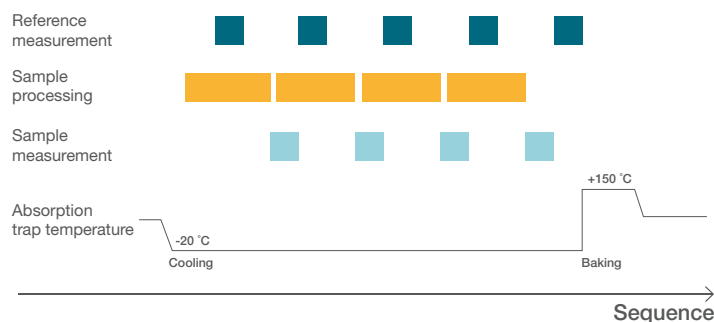


Figure 6. Graphical visualization of a sequence.

References

1. Eiler, J. M., (2007), Earth and Planetary Science Letters, 262, 309-327.
2. Eiler, J. M. (2011), Quaternary Science Reviews, 30, 3575-3588.
3. Kiel IV Carbonate Device Operating Manual.
4. 253 Plus 10 kV IRMS Operating Manual.
5. TN30668: LIDI 2 Software Workflow: Overcoming challenges for clumped isotopes CO₂ analysis on carbonate samples.
6. I. Müller et al.: Carbonate clumped isotope analyses with the long-integration dual-inlet (LIDI) workflow: scratching at the lower sample weight boundaries. Rapid Commun. Mass Spectrom. 2017, 31, 1057-1066. DOI: 10.1002/rcm.7878.
7. S. Bernasconi et al: Reducing Uncertainties in Carbonate Clumped Isotope Analysis Through Consistent Carbonate-Based Standardization. Geochemistry, Geophysics, Geosystems, 19. DOI: 10.1029/2017GC007385.

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