VERSION 1

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We are still developing and optimizing this protocol

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Carbon isotope discrimination - part II - gas analysis - v.01 V.1

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ABSTRACT

Protocol for measurement of carbon isotope discrimination during photosynthesis.

See the following references for more background:

H. Griffiths (1993) [Photosynthesis and Production in a Changing Environment](#) pp 181-1927

Farquhar et al. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Bioi.* 1989. 40:503-37

Start up the IRMS

- 1 Open inlet valve to vacuum (RL)
- 2 Switch between Ref and Sample (2x F1) Reference gas

Taking a reference-reference measurement

- 3 Purge reference lines
Open "RF"
Open "RP"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 4 Purge reference inlet
Close RF"
Open "RI"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

5 Isolate the reference lines and prepare for loading reference gas

Close 'RP'

Open 'RF'

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

6 Manually open valve and fill the reference loop with gas

Note

Make sure the second valve (top of image) is closed so gas only fills the reference loop. Open the first valve (depicted) for ~5s to fill collection loop.

7 Close the valve to the reference gas

- 8 Open the second valve, to let the reference gas from the connector piece into the inlet

Note

Make sure the first valve is closed, then open up the second valve (depicted) to let reference gas out of collection loop into the mass spec. You should see a change in the beam intensity as gas is detected. Wait until this stabilizes so all the gas has left the loop.

9 Close the second valve

10 Repeat steps 6-9 two times

11 Flood both sides of the device with reference gas
Close "LV" vacuum line
Open "RP"
Open "SP"
Open "SF"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 12 Isolate valves
Close "RF"
Close "RI"
Close "SF"
Close "SI"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 13 Check Beam 1
Look at the beam intensity, it should be $\approx 1 \times 10^{-12}$. You should expect a value of around 5×10^{-9}

Set beam intensity

- 14 V, then B : set target to 6×10^{-9} (Enter)

Note

This is to minimize the possibility of adding technical artifacts to measurements by always analysing gas at $\sim$ the same concentration

- 15 Do this for both Sample and Ref side

- 16 Manually adjust variable volume if the target beam intensity is below or above 6×10^{-9}

Note

Take small steps! adjusting the volume +/-5 is sufficient in most cases. The intensity will drift down over time as gas is consumed by the detector so always good to check both sample and reference immediately before measuring

Null reference as first run

- 17 D (Data processing) + sample name: "lrefref"
- 18 Cold finger: N
- 19 Run (make sure to feed new paper to the printer!)
- 20 Write values down in lab book
- 21 Open LVRun actual samples:

Load sample gas

- 22 Go to Sample side (use F1 F1 to switch from Ref to Sample)

- 23 Vacuum Sample side
Open "SP"
Open "SF"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 24 Check Bean 1 intensity => $\sim 1e-12$

Note

This is to check that the lines are clean, $1e-12$ is the value you get when there is little gas left in the system

- 25 Return Variable Volume Sample to 0;
V A (zero) 0

Note

This is done to reset the bellows for injection of the next sample.

- 26 Connect closed sample tube (SI closed)

- 27 Vacuum sample inlet
Close "SF"
Open "SI"
(SP already open)

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 28 Pirani gauge will go to $1e-3$, wait 10 more seconds

Note

The Pirani gauge gives an indication of the gas pressure in the inlet loop. A value of $1e-3$ means the loop is clean of gas so you are ready to inject.

- 29 Isolate from pump
Close "SP"
Close "SI"
Open "SF"

- 30 Carefully open collection tube (transducer value will rise)

Note

Software view. Diagram of valve system, arrow indicates an increase in signal from the Pirani gauge as gas build up behind valve SI

- 31 Load sample gas into system

Open 'SI'

Wait for beam 1 intensity to stabilize.

Note

Software view. Diagram of valve system, arrow indicates direction of gas flow. Beam 1 value will rise (if not, then only water in sample; close sample immediately and vacuum!)

- 32** Close off inlet
Close "SI"
Close "SF"

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

- 33** Adjust Beam intensity: V B 6e-9

- 34** Adjust reference beam intensity
Switch to Ref side (F1 F1)
V B 6e-9

Note

This is done due to drift in reference beam intensity as gas is used

Measure gas

- 35 Esc
menu D
sample ID
Cold finger: N
Make sure there is paper in the printer!

- 36 Note down values in lab book

Post-measurement

- 37 Vacuum Sample side
Open "SF"
Open "SP"
Return variable volume Sample to 0; V, A, 0

Note

Software view. Diagram of valve system, arrows indicate direction of gas flow

End of day

- 38 Open all valves on both sides except SI and RI

Note

This is done to clean out the system ready for the next use. Do not turn off the vacuum pump, it should run continuously to avoid wearing it out.

Access the latest run data

- 39 Esc (exit manual control menu)
- 40 F (Filer)
- 41 D (Data logger)
- 42 Select CO2: E (Edit current template)
- 43 Use cursor to scroll through to bottom of page for results (don't change anything!)
- 44 Esc to exit