

The Single-Step CO₂ Response (SSCO₂R™) Method

Rapid A/C_i Curves in Real Time Without Post Processing

The **Single-Step CO₂ Response (SSCO₂R™) Method** is a new high-speed ramping technique that eliminates all post processing and generates the data for A vs. C_i directly on the CIRAS-3 console in real time. Although initially designed for use with the CIRAS-4, we have built in the capacity to conduct the SSCO₂R™ Method with the CIRAS-3 as well!

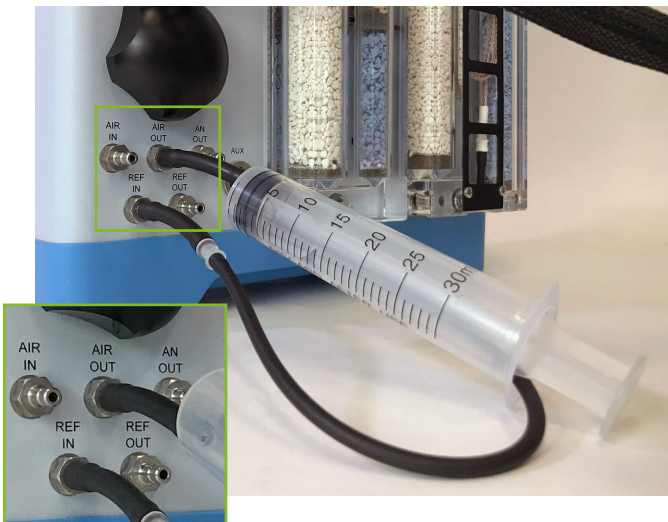
With The SSCO₂R™ Method, reference and analysis channels have identical time responses to a CO₂ ramp and delta CO₂ would be zero during an empty chamber ramp, eliminating the need for any corrections to A or C_i.

The SSCO₂R™ Method is the fastest, most accurate, streamlined method available for the rapid measurement of A/C_i — *more measurements and data points in a much shorter period of time!*

Set Up the CIRAS-3 and Record Data

1. Install the **Ramp Path Equalizer (STD581)**.

- i. Remove the black tubing from the gas ports on the back of the console. Fit the short piece of tubing from the **Ramp Path Equalizer** to the **AIR OUT** port and the long piece of tubing to the **REF IN** port.



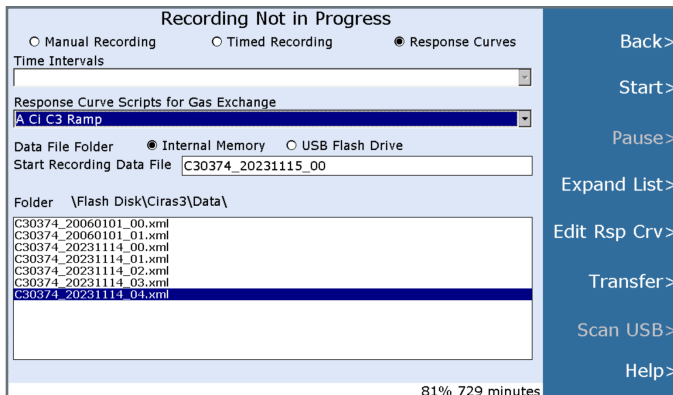
NOTE: Make certain the plunger is fully depressed on the **Ramp Path Equalizer**.

2. Perform a **Stored Diff Bal** calibration to allow the CIRAS-3 to have accurate offset information applied continuously throughout the linear ramp.

- i. Install fresh desiccants and a new CO₂ cartridge in the CIRAS-3 and allow the console to warm up.
- ii. Navigate to **Settings**. Set **Application** to **Gas Exchange** and change **Zero/Diff Bal Mode** to **Auto Zero/Stored Diff Bal**.
- iii. Navigate to **Operations > Calibration > Store Diff Bal** and press **Start**.
- iv. Once the calibration is completed (approximately 20 – 25 minutes), press **Accept**.

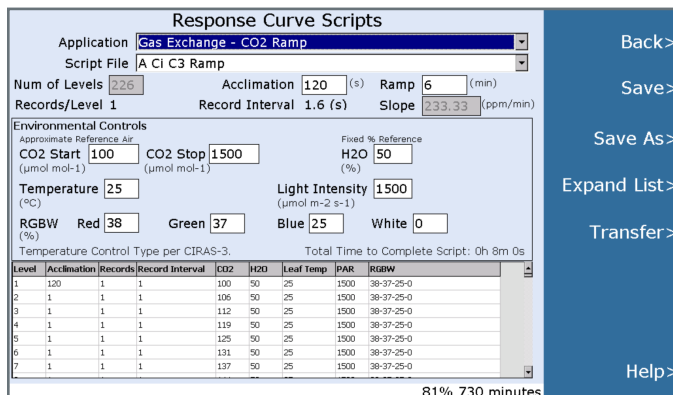
3. **Set the ramp criteria.** The ramp criteria is based on a response curve script. There are two standard scripts titled "A Ci C3 Ramp" and "A Ci C4 Ramp" available to download from ppsystems.com. Each script is designed for use with C₃ or C₄ plants. The script can be modified on the CIRAS-3 console or via the *CIRAS-3 Script Editing Software* (also available from ppsystems.com).

- Navigate to **Operations > Rec Options** and select **Response Curves**.
- In the dropdown menu below **Response Curve Scripts for Gas Exchange**, select the script (e.g., "A Ci C3 Ramp").



NOTE: If you have written a new script using the *CIRAS-3 Script Editing Software*, you must first upload the file via USB by selecting **Transfer** from the **Rec Options** screen. Next, choose the file you would like to transfer and select **Import**. Once complete, it will be available via the dropdown menu on the previous screen.

- If you wish to modify the script on the console, select **Edit Rsp Crv** from the **Rec Options** screen. Editing CO₂ settings will change the number of levels and the slope of the CO₂ ramp (ppm·min⁻¹). Feel free to change any additional settings from this screen (e.g., temperature, light intensity).

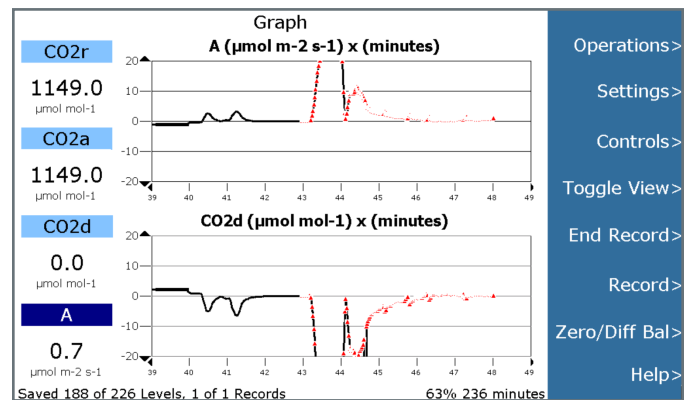


- Select **Start** from the **Rec Options** menu.

4. **With an empty and closed cuvette, adjust the volume of the plunger once the ramp has begun.** After an initial acclimation period, the CO₂r will increase at the rate set by the response curve. While CO₂r is increasing, observe a delay of CO₂a.

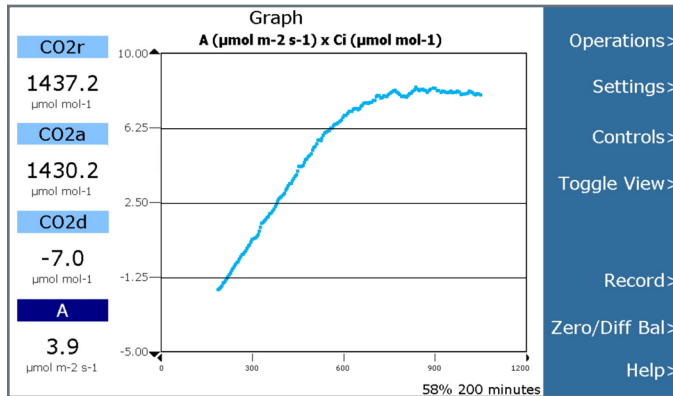
TIP: You can set a custom graph of CO₂d versus time to make this easier!

- Increase the volume of the plunger in 5 mL increments and observe the CO₂d approach 0. (Allow 20-30 seconds between adjustments to see this response.) Make adjustments as needed until the CO₂d stabilizes near 0 μmol·mol⁻¹. Once CO₂d stabilizes between -1 and 1, no further plunger adjustments are required and the path length of the Reference and Analysis IRGAs have been corrected. You may now end the ramp.

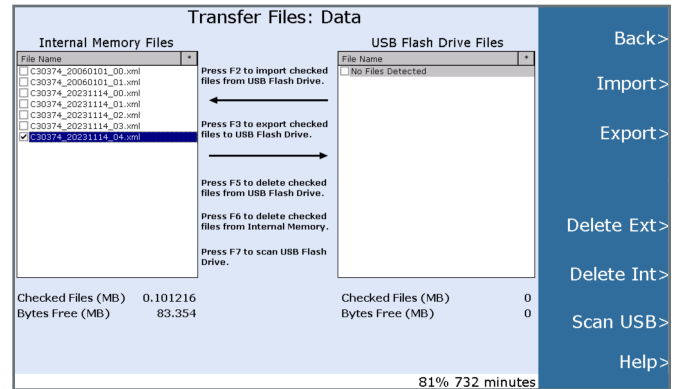


NOTE: In testing, the CO₂d typically appears to stabilize when the plunger volume is between 26 and 32 mL, although the exact volume may vary. Please also note that there may be some oscillation of CO₂d, which is to be expected, but should remain between -1 and 1.

5. Perform the ramp experiment with a leaf.
 - i. Repeat step 3 with a leaf positioned in the PLC3 Universal Leaf Cuvette chamber. If one watches the plot of A vs. Time (or A vs. C_i) for the ramp with the leaf, it becomes clear when CO₂ saturation occurs (a plateau), at which time the ramp can be terminated by selecting **End Record**.
6. Transfer data files to a PC.
 - i. Select **Transfer** from **Operations > Rec Options** and move the file from the console to a USB via the **Export** command.
 - ii. With the data open in Excel®, you may need to trim several rows at the beginning or end of the ramp to account for the response curve applying new settings.



Example of A vs. C_i plotted in real time directly on the CIRAS-3 console



NOTE: Once step 4 is complete, any number of ramp experiments can be performed on leaves without adjusting the plunger volume.

Please also note that adjusting the flow rate or changing the cuvette head plates will require a new stored diff bal (step 2) and plunger volume (step 4). Take care to avoid accidentally adjusting the plunger volume during or between measurements.



If you would like to learn more about this application or speak with one of our experienced technical staff, please feel free to get in direct contact with us via any of the contact information listed below:

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