
NATIONAL CORAL REEF MONITORING PROGRAM

Standard Operating Protocol

for

Dissolved Inorganic Carbon (Climate)

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NOAA
CORAL REEF
CONSERVATION PROGRAM

National Coral Reef Monitoring Program Dissolved Inorganic Carbon (DIC) Procedure

The AOML Coral Program team uses an Apollo SciTech, Model AS-C3 to measure (DIC) dissolved inorganic carbon in our seawater samples (Figure 1.). The Apollo machine measures DIC values in $\mu\text{mol/L}$, however our laboratory reports DIC values in $\mu\text{mol/kg}$. Therefore, the density of every sample is measured in order to convert to $\mu\text{mol/kg}$. Calibration is carried out using a brand-new (CRM) certified reference material bottle that has never been opened. The DIC machine needs to be calibrated every week or when a CRM is out of range. If the machine is already calibrated then a CRM sample needs to be run first to make sure that the calibration is still valid. CRM DIC values should read within $\pm 10\mu\text{mol/L}$ of the expected value of DIC for that batch of CRM. If not, then the machine needs to be recalibrated. See calibration procedure below for details.

If machine is already calibrated a CRM sample is always the first and last sample run on the machine. By running a CRM first our team can ascertain any possibilities for drift while running samples, and also allows us to calculate offsets.

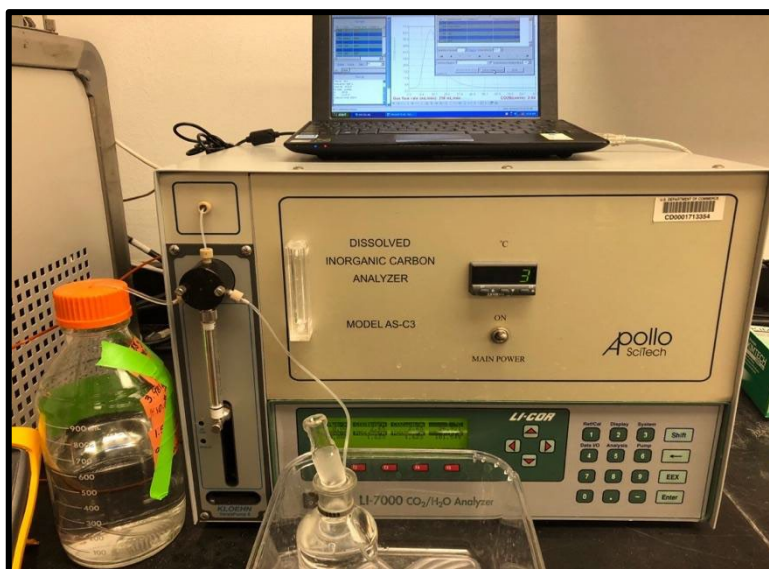


Figure 1. Apollo SciTech Dissolved Inorganic Carbon Analyzer-Model AS-C3. This machine measures DIC in $\mu\text{mol/L}$

How to run DIC Samples:

1. Turn on Main power switch. Machine needs to warm up for at least an hour before measurements are analyzed. This improves accuracy. Small screen displays the temperature (Figure 2), wait until the screen only reads numbers 4°C and below to begin measurements.



Figure 2. Temperature gauge on DIC machine. Wait until machine displays 4C and below to begin measurements.

2. Turn on the water bath on to the right of the DIC machine and place a new CRM (or a CRM that has only been used for DIC) into the bath. The temperature should read around 25°C (Figure 3).



Figure 3. Water bath set to 25C showing the appropriate water level.

3. Once the main screen reads 4°C and below consistently, turn on the bottom left switch on the Licor unit (LI-7000 CO₂/H₂O Analyzer), this will turn on the bottom screen.
4. Now turn on the Nitrogen tank to (~15 psi) (Figure 4).

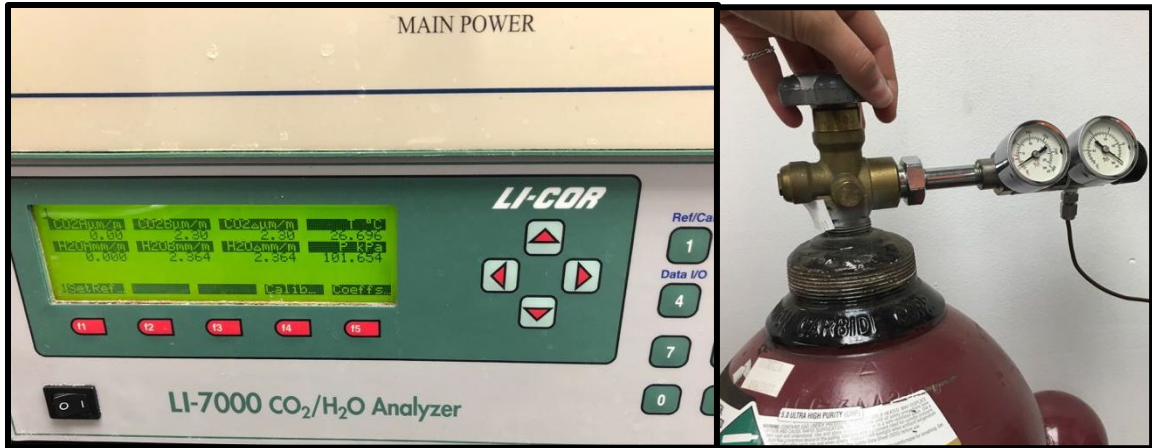


Figure 4. The photo on the left is a picture of the Licor screen active and ready. The photo on the right is the nitrogen on and at ~15(psi).

5. Power on the laptop above the machine, and navigate to the program called “DIC v2.36”.
6. Once the program opens select the small white paper icon at the top left of the program. This will connect/ disconnect the system (Figure 5).

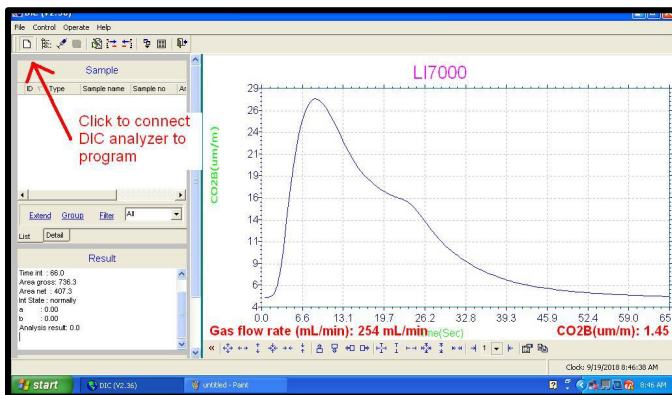


Figure 5. Screenshot depicting how to connect the DIC analyzer to the program.

7. Take the large hose to the left and place in DIC acid container. There should be a cap with a hole in the top, place hose through. Use the cap without the hole when system is not in use to prevent evaporation (Figure 6).

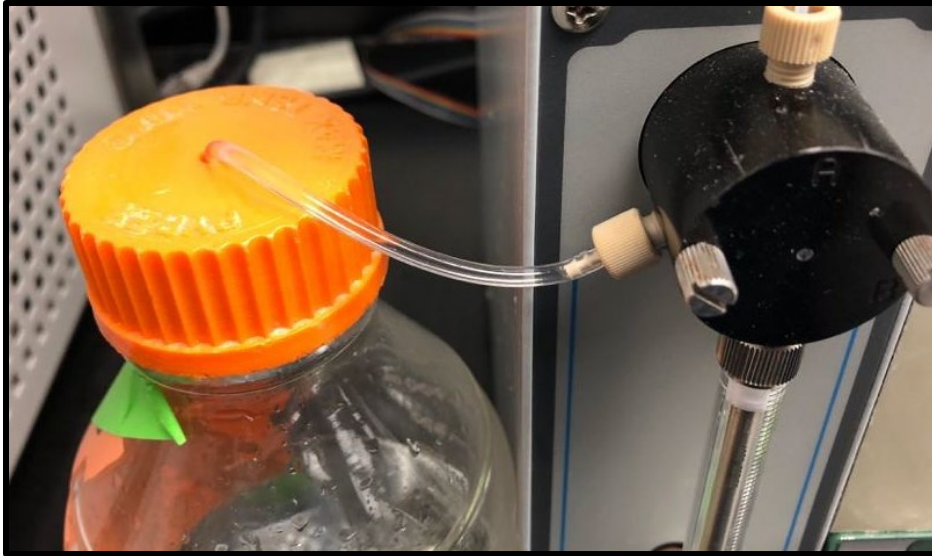


Figure 6. Small hose on left will be placed in DIC acid container.

8. The small hose to the right will be placed in the sample. Be sure to let the machine warm up for about an hour before proceeding with sample analysis.
9. Before starting a sample select the icon to the right of the white paper. This is the batch process icon. Once open a screen should appear with three tabs.
10. Select the “Sample list” tab. To add a new sample, select the + sign. This will add two new columns to the samples list, it is a glitch in the software. Delete the last column by selecting the – sign while the last column is highlighted.
11. In the “Sample Name” column, input either the bottle letter ID (ex. 12A) or the bottle tag number on the ziptie (ex. 2124). (Figure 7).

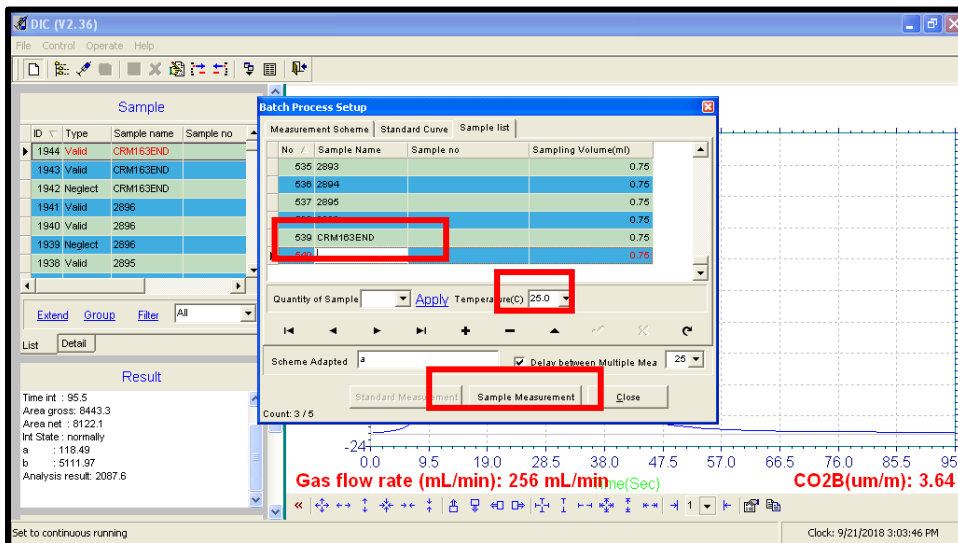


Figure 7. Screenshot of how to enter a new sample and where to click to begin the sample measurement.

12. Now take the sample out of the water bath. Use the thermometer and record the temperature of the sample, sample should be 25°C. Insert the small hose on the right side of the pump (Figure 8). Then place the cap of the bottle light on the neck of the bottle to minimize CO₂ fluctuations between the sample and air. Enter the temperature in the box on the sample list screen.
13. Once the temperature, sampling volume (should be 0.75mL automatically), and sample name have been entered, hit “Sample measurement” at the bottom. This will start the sample. Repeat these steps for all samples.
14. Once the sample has been completed its data can be exported. Select the “test results” icon (it looks like a paper and pencil). This will open all of the data. (Figure 9).

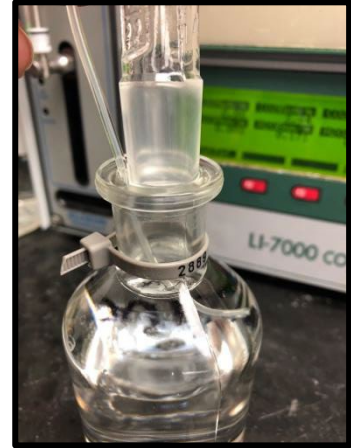


Figure 8. Proper placement for DIC hose in sample.

ID	Type	Sample name	Sample no	Type	Analysis time	Spl volume(ml)	Analysis result	Area net	Temperature(C)	a	b
1865	CRM163START			Neglect	9/21/2018 9:33:10 AM	0.75	1861.2	6487.4	25.0		118.49
1866	CRM163START			Out of d	9/21/2018 9:36:11 AM	0.75	2053.2	7990.5	25.0		118.49
1867	CRM163START			Valid	9/21/2018 9:39:12 AM	0.75	2084.7	8111.2	25.0		118.49
1868	CRM163START			Valid	9/21/2018 9:42:14 AM	0.75	2094.1	8108.7	25.0		118.49
1869	2873			Neglect	9/21/2018 9:54:47 AM	0.75	2216.3	8615.9	25.0		118.49
1870	2873			Valid	9/21/2018 9:57:46 AM	0.75	2319.5	9011.5	25.0		118.49
1871	2873			Valid	9/21/2018 10:00:43 AM	0.75	2319.0	9009.5	25.0		118.49
1872	2874			Neglect	9/21/2018 10:04:06 AM	0.75	2269.7	8897.3	25.0		118.48
1873	2874			Valid	9/21/2018 10:07:04 AM	0.75	2286.1	8883.5	25.0		118.49
1874	2874			Valid	9/21/2018 10:10:02 AM	0.75	2287.5	8888.9	25.0		118.49
1875	2875			Neglect	9/21/2018 10:25:42 AM	0.75	2082.0	8100.9	25.0		118.49
1876	2875			Out of d	9/21/2018 10:28:40 AM	0.75	2068.8	8054.4	25.0		118.49

Figure 9. Screenshot of test results. Record two valid values for each sample measurement.

15. When the sample is complete, the box labeled sample on the upper left will contain the data. If the sample run is satisfactory the box should contain a neglect and two valid readings for the sample.
16. It is not uncommon for there to be one or more Out of D readings. As long as there are two valid readings for that sample the data is good. If there aren't two valid readings the sample needs to be redone.
17. Record the two valid DIC values into the DIC record book, along with the temperature recorded of the sample. (Figure 10).

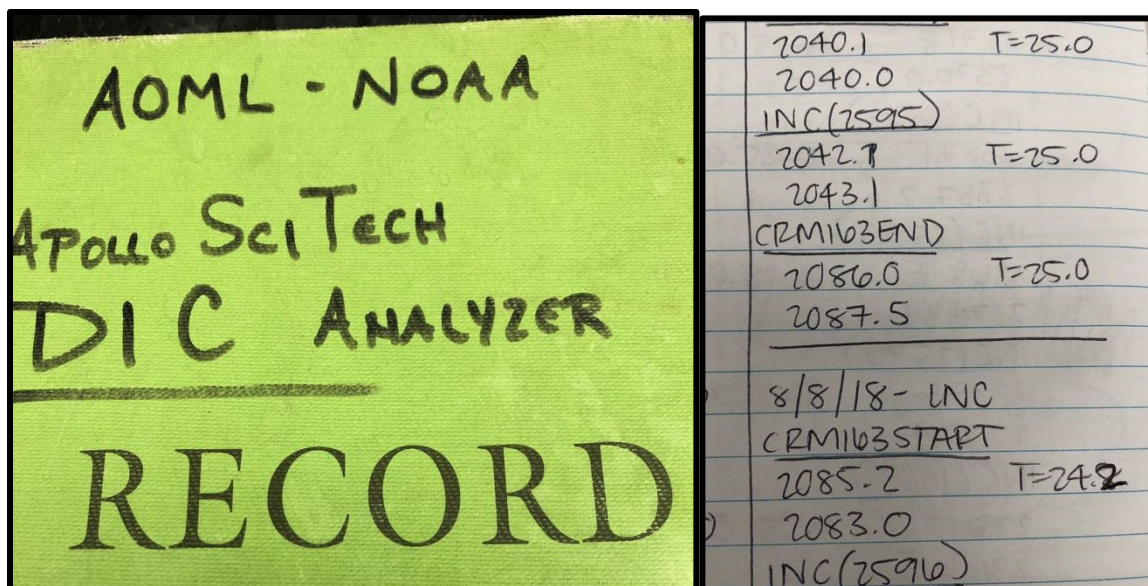


Figure 10. Photo of the DIC record book as well as how to properly record the data.

18. When you are finished with the DIC machine AND HAVE RUN THE SAME CRM YOU USED TO START WITH, place both hoses into DI water. Replace cap the proper cap on the DIC acid container.
19. Select the white paper connect/ disconnect icon in the top left corner of the program.
20. Confirm stop of the experiment and hit “ok”.
21. Flush with the DI water and hit “ok”.
22. Once the DI water has been flushed, turn off the bottom switch to the LI-COR first, followed by the top switch.
23. Next turn off the nitrogen.
24. Dispose of acid waste by diluting solution and pouring into the hazmat disposal container.
25. Finally turn of the water bath.

How to calibrate the DIC machine:

1. See steps 1-7 above. Once machines are on, follow these directions.
2. Place a fresh, unopened, bottle of CRM in the water bath once on.
3. Before starting a sample select the icon to the right of the white paper. This should be the batch process icon. Once open a screen should appear with three tabs.
4. Select the tab “standard curve”. Delete the value in the standard concentration ($\mu\text{mol/L}$) box. Select Reset to delete the old standard readings. The Area net should now be clear (Figure 11).
5. A three-point calibration is used. Each sample is measured at 0.75mL, therefore we do a linear three-point calibration with values of 0.60mL, 0.75mL, and 0.80mL. These should automatically show when program is opened.

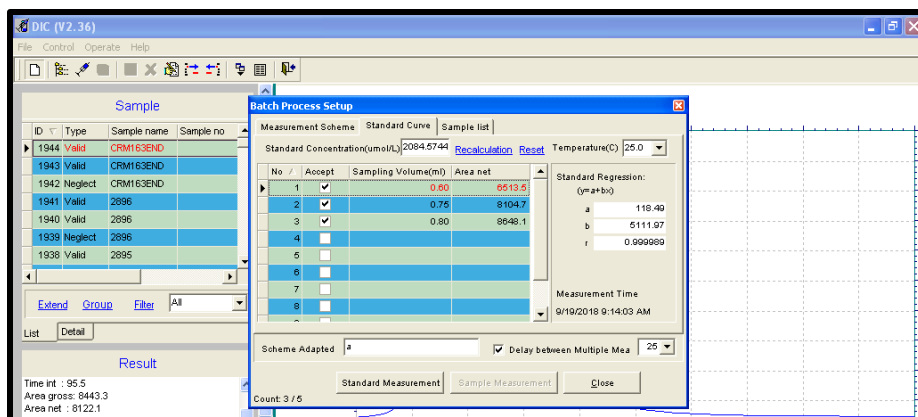


Figure 11. Calibration process window where the calibration details are entered.

6. Enter the CRM name and salinity calculated from the density calculations spreadsheet located on the Desktop of DIC computer.
7. Take the CRM out of the water bath and record the temperature - ideally, we want to measure at 25°C, keep in water bath until 25°C is reached (~30/40mins).
8. Go to the desktop and open Density calculations (Figure 12).
 - a. Drag down the density calculation
 - b. The DIC $\mu\text{mol/kg}$ depends on the CRM batch used (Standard certified values of CRMs found at https://www.nodc.noaa.gov/ocads/oceans/Dickson_CRM/batches.html)
 - c. Drag down last column to convert the DIC $\mu\text{mol/kg}$ to DIC $\mu\text{mol/L}$
 - i. This is the number that will be entered in the standard curve standard concentration box

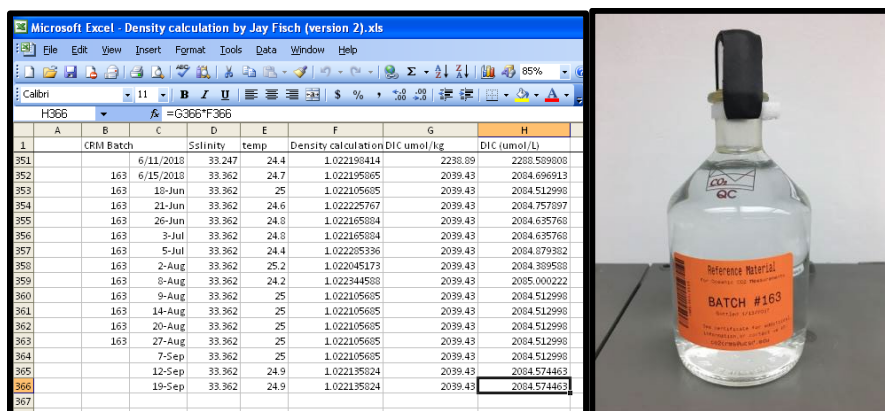


Figure 12. The excel spreadsheet used to calculate appropriate DIC settings for calibration. The bottle on the right is called a CRM-Certified Reference Material

9. Copy and paste this number to the DIC program. Change the temperature to the temperature recorded in the density calculation. “Click recalculate”

10. Then make sure the small hose to the left of the pump is in the CRM sample and the larger hose to the right is in the acid.
 - a. Hit standard measure. This will start the calibration.
 - i. Once complete each sampling volume will have a new area net
 - ii. This changes the A and B values used in the calculations
11. Once calibration is complete normal sampling can be performed. But first a CRM must still be run even after a calibration has been completed.

Last reviewed: April 21, 2023