Updated: 08-24-22 SJW
05-20-25 IVB

SERC: Seal Analytical AQ300 Protocols

START-UP PROCEDURES

Turn the instrument on.

- The power button is on the back-right side of the instrument just above the power cord.
- There is also a lamp only switch higher up on the back of the instrument so make sure the proper switch is used.

Log onto the computer and open the AQ300 software.

- Do not open the software without the instrument being on. The software does not like this.
- Log in name is “seal”, password is “toledo”. A checklist will pop up.

Take out reagents & samples

- to allow them to get to up to temp

Perform the daily checklist items:

- ☐ Empty and refill the wash water reservoir and DI reagent
    - *Even if it’s full, still get fresh DI every day*
    - *Tap sides to remove bubbles*
- ☐ Empty the waste container
    - Make sure the waste carboy is not full and labeled correctly for hazardous waste
    - Log in sheet and contact the proper personnel if full
    - This should ideally be transferred to a closed-top container after every use
- ☐ Replace reaction segments
    - Maintenance & Setup Tab -> Maintenance
        - *The sash can be open, but it will move, look for smooth movements*
    - The instrument will then initialize
        - Observe movements during initialization
            - Movements should be smooth and they should be the same each initialization.
    - To check if reaction segments need to be replaced (shouldn’t be the case)
        - Initialize -> Zero Segments
            - *The pop-up should say all reaction segments are clean.*
            - *If not, replace the ones it indicates.*

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- ☐ Syringe Prime and Probe Washer Check
    - Maintenance and Setup Tab -> Diluter
        - Prime syringe 10 times (change number on drop-down list).
            - *Verify that there are no bubbles/air in the syringe by the end, and it is operating smoothly*
        - Turn on the waste pump and wash valve.
            - *Check for leaks*
            - Verify a vortex in the chamber and no water dripping.
        - **Turn off** the wash valve and waste pump.
- ☐ Cuvette and Washbath Check
    - Maintenance and Setup Tab -> Cuvette
        - Perform 5 auto washes (change number on drop-down list).
        - Observe wash bath is filling and clean.
            - *Bath is in the back; look for water on the probe*
    - Check/Adjust aspiration for both inner and outer wells (run check twice)
        - Verify that there is 1-2" of water (no bubbles) in the outlet tubing of cuvette.
            - *You don't want any bubbles close to the cuvette, but further up the tubing is okay.*
            - *REMEMBER TO PUT THE COVER BACK ON THE CUVETTE!!!!*
        - If you need to adjust, change the value in increments of ± 5.
        - This number is essentially pump rotations.
        - You may also need to replace the tubing if large adjustments are needed OR if the pump is making a lot of noise.

Close out of the maintenance window.

Make sure there is DI in reagent space 18 and cuvette cleaner in reagent space 1

Run the "Daily Startup" procedure and click continue when prompted.

- This will take just a minute or two.

Once the small daily startup window disappears, click on "print preview."

- Check that the gains from the last time the startup was ran (left columns) are not wildly different from current gains (right columns).
- If the numbers are quite different, there might be a problem with the lamp, and refer to the customer support manual.
- You can close the print preview window after comparing.
    - *Replace the DI in space 18 every day as well*
    - *Cuvette cleaner is in the drawer if you need to refill it*

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RUNNING THE ANALYSIS

Click the Scheduling Tab

Choose a free tray and choose what reagent tray is appropriate for the methods you are running.

Rename with your initials and the number of trays for the day you are doing.

- (IVB-1) if it was the first run of the day
- If making trays for future runs, label with more information for ease of selection

Click "Show stds/ctls" after the new scheduling tray appears

- Do not place any samples in the red highlighted rows.
- *If running V-NO_X and NH_3/PO_4 in the same day, do not start until cup 5

You can now type in or import your samples into the sample ID column.

- PW Samples should be named as follows:
  - Site_YYYYMM_ZONE_LYSA/B/C_Depthcm
    - Ex. GWI_202208_LysA_10cm
    - Ex. GCrew_202208_LysC_45cm
- Rhizon Samples should be named as follows:
  - Site_YYYYMM_RHZ_ZONE_SF_#
    - Ex. MSM_202208_RHZ_TR_SF_1
    - Ex. MSM_202208_RHZ_UP_SF_7

You should insert a dup and spike every 10 samples.

- To do this, highlight the row after the sample in cups 10/11.
- Add a row and make the sample type dup/spkA
- Click cup number, pulldown, and click on the same cup number (10/11)
  - This will assign these rows the same cup as your dup/spike sample.
- Repeat every 10 samples.

You should add artificial seawater to account for matrix effects

- Add the artificial seawater using the naming scheme
  - Abs_Chk_Xppt (X = concentration used)
- Add an Auto Spike
  - insert a row below, set sample type to SpkA and make the cup number the same as the artificial seawater
- Recipe
  - 10 ppt artificial seawater
    - 10 g NaCl
    - 0.2 g NaHCO3
    - In 1000mL DI water
  - 20 ppt artificial seawater
    - 20 g NaCl
    - 0.2 g $NaHCO_3$
    - In 1000 ml DI water

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Test Dependent Steps

- NO_x Vanadium Run
  - Sample Cup #1 -> Top Standard 1ppm
  - Sample Cup #2 -> Nitrate Reduction (NO_3^-) Efficiency Check
    - switch sample type in cup 2 to "Ref", which will fill in cup 2 & 3
  - Sample Cup #3 -> Nitrite (NO_2^-) Reduction Efficiency Check
  - Sample Cup #4 -> Leave Blank
  - End of run (after artificial seawaters) peCheck 1.51 ppm Nitrate
    - **Check the PECHK_Concentration file in the SEAL team folder
    - Sample name: "peChk_NOx"
  - Reagents Needed (Check by going to the Maintenance tab -> Test -> Reagent Tab)
    - 1. Cuvette Cleaning Solution
    - 2. V-NO_x Color Reagent
    - 10. V-NO_x CCBV 0.5 ppm NO_3^-
    - 11. V-NO_x Spike 5 ppm NO_3^-
    - 18. DI Water (fresh daily)
- NH_3 & PO_4
  - Sample Cup #1 -> PO_4 Top Standard = 0.3ppm
  - Sample Cup #2 -> NH_3 Top Standard = 2ppm
  - Sample Cup #3&4 -> Leave Blank
  - End of run (after artificial seawaters) peCheck 1.034 ppm NH_3 and 0.824 ppm PO_4
    - **Check the PECHK_Concentration file in the SEAL team folder
    - Sample name: "peChk_NH3_PO4"
  - Reagents Needed (check SEAL Reagents Sheet for Expiration)
    - 1. Cuvette Cleaning Solution
    - 2. Phosphate Color Reagent
    - 3. Ascorbic Acid (made fresh daily*)
    - 4. Regular EDTA Solution
    - 5. Alkaline Phenate
    - 6. Hypochlorite (Bleach)
    - 7. Nitroferricyanide
    - 9. OP CCV: 0.15 ppm PO_4
    - 10. NH_3 CCV 1 mg/L NH_3
    - 13. NH_3 Spike Solution 4ppm N
    - 15. PO_4 Spike Solution 4ppm P
    - 18. DI Water (fresh)

  Ascorbic Acid Recipe

  - 100ml DI H_2O & 1 mg Ascorbic Acid

Assign a test and save once all your samples are entered

- Highlight all the rows and click the analysis you want to run from the test box and save

Be sure standards and controls are also in the correct cup or reagent and the lid is closed.

Double click "Run" and select the tray to begin the analysis.

- *You want to select "Auto Calibration" to do a standard curve*
- *Turn on "*auto turn lamp off*", especially if you have a long run into the evening.*
- *Turn on "Water Baseline"*
- *If a full run, replace the first reaction segment and check that box*

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SAMPLE STORAGE

Combine the run samples into a 1 or 2 gal plastic bag

Label

- COMPASS: SYNOPTIC CB

  NON-HAZ POREWATER

  NUTRI: FILTERED & FROZEN

  MONTH/YEAR

- CONTACT: S. WILSON

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  Biogeochem Lab

- Run: ✓ NO_x ✓ NH_3/PO_4

  Revisit: Month/Year + 2/3

Store in the appropriate bin in the walk-in freezer in the basement

DATA RESULTS

Once the run has finished:

- Tray Manager -> Select correct tray (from list)- > Tray Log (on top)->
- In the new popup window select, Ascending order -> Copy in Excel Format
- Open Excel and paste data.
  - *Column F: Concentration*
  - *Column G: Absorbance*
  - *Column I: Dilution*
- Files should be named as follows:
  - SEAL_COMPASS_Synoptic_Analysis_MonYYYY_#
    - Ex. SEAL_COMPASS_Synoptic_NOx_Aug2022_2
    - Ex. SEAL_COMPASS_Synoptic_NH3_PO4_Aug2022_1
- Save the file to the appropriate folder as a csv
    - Ex. SEAL_COMPASS_Synoptic_NH3_PO4_Aug2022_2
- *In the QA/QC log write the slope, intercept, and R2 of the standard curve*

Shut Down Procedure

Remove the sample cup tray from the instrument and dispose of the sample properly.

- Rinse with DI and empty into haz waste.
- Plastic cups and reaction wells can go in trash once emptied.

Make sure DI is placed in Reagent 18 slot and cuvette cleaner is placed in Reagent 1 slot.

Close the lid.

Maintenance & Setup Tab -> Maintenance -> Cuvette -> Extra Wash

Extra wash will take a few minutes.

Cap and place reagents in the fridge.

Remove used reaction wells and rinse and dispose of properly (hazardous waste).

- To see which reaction wells to replace:
    - Initialize -> Zero Segments
    - Pop up should indicate which wells to replace with new ones.
    - Check that new wells are free from dust & dirt.

Click "Ok" once they are replaced.

Close out of the maintenance window and close out of the software.

Turn the power off to the instrument from the back button just above the power cord.

GENERAL NOTES

- There is a USB on the table that has all the protocols and software on it if you ever need it.
- NH4/NH3 concentrations can be affected by freeze/thaw so you want to run these when you thaw the samples – prioritize this if you can only run one thing after thawing a set of samples.
- If you run samples for NOx first, you can just top off the samples and run them for NH4/PO4 directly after to save time pouring
- If you have samples that seem to have particles in them, you need to refilter into the sample cup with a 0.45 uM filter – please make a note of this in the sample log.
- CCV/CCBs will run automatically every 10 samples
- If the sample is brown/grey in color, you may need to dilute it
    - First step is running it with an auto spike to check that it clears this
    - You can add a dilution line to this sample in the software if needed
    - *The machine should auto blank every sample so this should not usually be an issue.*