RNA Extraction using RNAqueous-4PCR Kit for Diatoms

Preparation

1. Remove samples from -80°C freezer and let thaw (Once thawed, put on ice)
2. Preheat elution solution to 75°C using heat block
3. Clean bench space and forceps using 70% ethanol
4. Collect autoclaved tubes needed for each sample and set under UV lamp for 15 minutes before starting the extraction. Use forceps to insert the tubes into the holder. Materials needed for each sample are:
   1. 1-1.5mL collection tubes
   2. 1-800µL collection tube  
      Also, 1-1.5mL collection tubes for temporarily holding the filters (not UV’d)

RNA Isolation Procedure

1. Insert 500µL of the Lysis Buffer to the sample under the hood.
2. Insert glass beads into each sample using a plastic spatula.
3. Place the sample tube into the bead beater. Turn on the bead beater at a speed of 48 for one minute (6).
4. Centrifuge tubes for 1 minute at 8,000rpm or longer if foam persists.
5. Remove filters from tubes using forceps and cleaning forceps with 70% ethanol between tubes.
6. Centrifuge tubes for 1 minute at 8,000rpm or longer if foam persists.
7. Decant off the Lysis buffer from bead solution by pipetting and put into one of the 1.5mL collection tubes.
8. Add an equal volume of 64% ethanol to the lysate and mix by inverting, flicking, and vortexing (on setting 6).
9. Insert a Filter Cartridge into the provided Collection Tube using forceps. Set up one of these for every collection tube containing the Lysis solution.
10. Apply the lysate/ethanol mixture from Step 8 to the corresponding Filter Cartridge and Collection Tube.
11. Centrifuge for 1 minute at 14,000 rpm then discard the flow through.
12. Repeat steps 10-11 until all of the sample has been filtered.
13. Apply 700µL Wash Solution #1 to the Filter Cartridge.
14. Centrifuge for 1 minute at 14,000 rpm then discard the flow through.
15. Add 500µL Wash Solution #2/3 to the Filter Cartridge.
16. Centrifuge for 1 minute at 14,000 rpm then discard the flow through.
17. Repeat Steps 15-16 for each collection tube.
18. Put the Filter Cartridge into a fresh Collection Tube.
19. Pipet 40µL preheated Elution Solution to the center of the filter. Close the cap of the tube.
20. Recover eluate by centrifugation for 1 minute at 14,000 rpm.
21. Pipet 20µL preheated Elution Solution to the center of the filter. Close the cap of the tube.
22. Recover eluate by centrifugation for 1 minute at 14,000 rpm.
23. Discard filters.

DNase 1 Treatment and DNase Inactivation

1. Remove DNase 1 Buffer from fridge and allow to thaw
2. Add 0.1 volume (6µL if total volume=60µL) of 10X DNase 1 Buffer
3. Add 2µL of DNase 1 by inserting the tip of the pipet into the liquid solution
4. Mix gently by flicking (possibly gently vortexing), then give the sample a quick spin.
5. Incubate for 45 minutes at 37°C in the MaxQ shaker.
6. Remove DNase Inactivation Reagent from fridge and flick, and vortex.
7. Cit ¼ length off the end of a 10µL pipet tip.
8. Add 0.1 volume (6µL if total volume=60µL) DNase Inactivation Reagent, then flick to disperse reagent.
9. Mix gently, incubate 2 minutes at Room Temperature
10. Centrifuge at 10,000 x g for about 1 minute to pellet the DNase Inactivation Reagent
11. Remove RNA using pipet and place in new tubes that have been UV’d and labeled.