RNA Purification from Zooxanthellae using the Epicentre MasterPure Purification Kit

1) Determine cell density of culture using a hemocytometer. Transfer approximately 3 X 10^6 cells to a clean 1.5 mL eppie tube. Pellet cells by centrifugation (approximately 3 min).
2) Remove f/2 media and replace with ddH_2O. Vortex and pellet cells again by centrifugation. Remove all but 25 μL of the ddH_2O.
3) Vortex for 10 sec to resuspend cell pellet.
4) Add 300 μL Tissue and Cell Lysis Solution and baked glass beads to tube.
5) Vortex for 15 min to break cells.
6) Add 1 μL of 50 μg/μL Proteinase K to each tube.
7) Incubate at 65°C for 30 min; vortex every 5 min.
8) Place samples on ice for 3-5 min.
9) Add 150 μL MPC Protein Precipitation Reagent to each sample and vortex for 10 sec.
10) Pellet debris for 10 min. If pellet is clear, small or loose, add 25 μL of MPC Protein Precipitation Reagent, mix, and pellet debris again.
11) Transfer supernatant into new tube.
12) Add 500 μL isopropanol alcohol and invert 30-40X.
13) Pellet RNA by centrifugation at 4°C for 10 min.
14) Carefully pour off isopropanol without dislodging pellet. Carefully remove all of the isopropanol with pipet.
15) Add 200 μL of DNAse I Solution to each tube. Dilute 5 μL of DNAse I enzyme into each tube. Make sure pellet is completely dissolved.
16) Incubate at 37°C for approximately 60 min.
17) Add 200 μL of 2X T & C Lysis Solution; vortex for 5 sec.
18) Add 200 μL of MPC Protein Precipitation Reagent; vortex 10 sec; place on ice 3-5 min.
19) Pellet debris by centrifuging for 10 min.
20) Transfer supernatant into new tube.
21) Add 500 μL isopropanol alcohol and invert 30-40X.
22) Pellet RNA by centrifugation at 4°C for 10 min.
23) Carefully pour off isopropanol without dislodging pellet.
24) Rinse twice with 75% EtOH, being careful not to dislodge pellet. If pellet becomes dislodged, centrifuge briefly. Remove residual EtOH with pipet.
25) Resuspend RNA pellet in 30 μL TE Buffer.
26) Quantify by running 5 μL in a 2% TBE agarose gel.