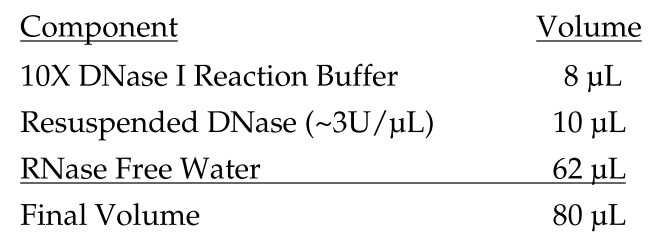
**Larvae and primary polyps RNA extraction**

1. Add 550 uL trizol to the larvae and homogenize.
2. Put homogenized (550 uL) into a qiashredder column and centrifuge for 1 minute (12000g).
3. Add 450 uL trizol to the collection tube.
4. Incubate 5 minutes at 15°-30° C.
5. Add 0.1 ml BCP
6. Vortex 15 seconds and incubate at 15°-30° C for 2-3 minutes.
7. Centrifuge at 12000g for 15 minutes.
8. Transfer supernatant to a RNA-free tube and follow Invitrogen **RNA mini kit** (page 27 of the manual) or the Invitrogen **RNA micro kit**:
9. Add equal volume (~ 0.6 ml) of 70% ethanol to the tissue homogenate.
10. Mix thoroughly by shaking or vortexing to disperse any visible precipitate that may form after adding ethanol.
11. Transfer ≤700 μL of the sample (including any remaining precipitate) to the Spin Cartridge.
12. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
13. Repeat Steps 11–12 until the entire sample is processed. If DNA-free total RNA is required, proceed to On-column PureLink® DNase Treatment Protocol (page 63).
14. Prepare PureLink ® DNase by adding the following components (supplied with PureLink ® DNase) to a clean, RNase-free microcentrifuge tube. Prepare 80 μL per sample.
15. 
16. Add 350 μL Wash Buffer I to the Spin Cartridge containing the bound RNA. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube.
17. Add 80 μL PureLink ® DNase mixture (prepared as described above) directly onto the surface of the Spin Cartridge membrane.
18. Incubate at room temperature for <15 minutes.
19. Add 350 μL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 x g for 15 seconds at room temperature. Discard flow-through and the Collection Tube and insert the Spin Cartridge into a new Collection Tube.
20. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
21. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
22. Repeat Steps 20-21 once.
23. Centrifuge the Spin Cartridge at 12,000 × g for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
24. Add 100 μL RNase-Free Water to the center of the Spin Cartridge.
25. Incubate at room temperature for 1 minute.
26. Centrifuge for 2 minutes at ≥12,000 × g at room temperature.
27. Proceed to analyzing RNA Yield and Quality (Nanodrop and TapeStation).
28. Transfer supernatant to a RNA-free tube and follow Invitrogen **RNA micro kit** (page 19):
29. Add one volume (0.6 ml) of 70% ethanol to the tissue homogenate.
30. Mix thoroughly by shaking or vortexing to disperse any visible precipitate that may form after adding ethanol.
31. Transfer ≤700 μL of the sample (including any remaining precipitate) to the Spin Cartridge.
32. Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
33. Repeat Steps 11-12 until the entire sample is processed.
34. Add 350 μL Wash Buffer I to the Spin Cartridge. Note: if Dnase not performed, use 600 ul of wash buffer I, centrifuge at 12,000 × g for 30 seconds at room temperature. Discard flow-through and proceed to step 21.
35. Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube.
36. Add 10 ul reconstituted purelink Dnase to 10 ul 2x Dnase buffer to obtain a 20 ul mixture. Mix by pipetting up and down.
37. Add 20 ul Dnase mixture to the center of the column.
38. Incubate at room temp. for 15 minutes.
39. Add 350 ul Buffer I to the center of the column.
40. Centrifuge at 12,000 × g for 15 seconds at room temperature.
41. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
42. Centrifuge at 12,000 × g for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
43. Repeat Steps 21-22 once.
44. Centrifuge the Spin Cartridge at 12,000 × g for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
45. Add 12-22 μL RNase-Free Water to the center of the Spin Cartridge.

Note: The dead volume of the PureLink™ Micro Kit Column is ~2 μl. An elution volume of 12 μl will result in a final elute volume of 10 μl.

1. Incubate at room temperature for 1 minute.
2. Centrifuge for 1 minutes at ≥12,000 × g at room temperature.
3. Store your purified RNA (see page 4), or proceed to Analyzing RNA Yield and Quality