**Note:** Prior to using a new kit, add 10.5 mL of 100% molecular grade ethanol to the bottle labeled Wash Soln 1 Concentrate and mix well. Place a check mark in the empty box on the label to indicate that the ethanol has been added.

**Note:** Prior to using a new kit, add 22.4 mL of 100% molecular grade ethanol to the bottle labeled Wash 2/3 Concentrate. Place a check mark in the empty box on the label to indicate that the ethanol has been added.

**Sample Preparation**

1. For each sample, label a 2.0 mL tube, two 1.5 mL tubes, a Micro Filter Cartridge Assembly, and two 0.6 mL tubes.
   1. Fill bottom cone of 2.0 mL tubes with a minimal amount of 0.5 mm glass beads.
   2. UV sterilize all tubes except filter tubes.
   3. Add 200 μl of lysis buffer to the bead tubes and place tubes on ice.
2. Thaw samples on ice in the hood. Prep tissue collection station with sterile razor blades, Kimwipes, ethanol, and forceps.
3. Prep reagents:
   1. Warm Wash Solution 2/3 to room temperature.
   2. Heat aliquots of Elution Solution in 2.0 mL tubes in heat block at 75°C. The volume needed depends on the number of samples. Each sample is eluted in 20 μl, however, you should heat at least 25 μl of Elution Solution per sample.
   3. If continuing through DNase treatment, thaw 10X DNase *I* Buffer and DNase *I* on ice.
4. Using a new sterile scalpel blade per sample, scrape tissue out of one polyp from the fragment and into the bead tube.
5. Beat-beat tubes at 5 m/s for 1 min. Keep on ice before and after.
6. Centrifuge at 16.5 × g for 2 min. Tap tube on bench until foam settles and spin for another 2 min.
7. Transfer ~100–150 μl of supernatant to a 1.5 mL tube, being careful to avoid pipetting beads, skeletal debris, and mucus. These will clog the filters.
8. Centrifuge at 16.5 × g for 2 min. Transfer supernatant to a new 1.5 mL tube.

**RNA Extraction**

1. Add 1.25 volumes of 100% ethanol (150 μl) to each tube and mix by back pipetting. Remove any remaining mucus from the tubes using the pipette tip.
2. Transfer the lysate to a filter tube and centrifuge at 16.5 × g for 2 min.
3. Add 180 μL of Wash Solution 1 to the filter and centrifuge at 16.5 × g for 2 min. Pour out the flow-through as needed.
4. Add 180 μL of Wash Solution 2/3 to the filter and centrifuge at 16.5 × g for 2 min.
5. Add a second 180 μL aliquot of Wash Solution 2/3 to the filter and centrifuge at 16.5 × g for 2 min. Pour out the flow-through as needed.
6. Centrifuge at 16.5 × g for 2 min to dry the filter.
7. Open tubes and place on ice. Place a Kimwipe over tubes and allow to dry for 2 min.
8. Transfer the filter to an Elution Tube.
9. Apply 20 μL of Elution Solution, preheated to 75°C, to the center of the filter. Let sit for 1 min at room temperature, then centrifuge at 16.5 × g for 1 min.
10. Repeat elution with same 20 μL of Elution Solution and centrifuge at 16.5 × g for 1 min.
11. Transfer eluted RNA to a 0.6 mL tube.

**DNase *I* Treatment**

1. Prepare a master mix using the volumes needed for each sample:
   1. 1/10 volume 10X DNase *I* Buffer (2.0 μL for RNA eluted in 20 μL)
   2. 1.0 μL of DNase *I*
   3. 24 samples will fit in the heat block, so prepare master mix for 26 samples (52 μL of DNase Buffer, 26 μL of DNase *I*).
   4. Mix gently but thoroughly.
2. Add 3.0 μL of master mix to each sample. Mix gently by back pipetting.
3. Incubate the DNase reaction for 30 min at 37°C. Remove the DNase Inactivation Reagent from –20°C and allow it to thaw at room temperature during this incubation.
4. Vortex the DNase Inactivation Reagent vigorously to completely resuspend the slurry.
5. Add 2.0 μL or 1/10 volume (2.0 μL for RNA eluted in 20 μL) DNase Inactivation Reagent, whichever is greater. Flick to mix.
6. Store the reaction at room temperature for 2 min, flicking once during this interval to disperse the DNase Inactivation Reagent.
7. Centrifuge the reaction at 16.5 × g for 2 min to pellet the DNase Inactivation Reagent. Transfer the supernatant to a fresh 0.6 mL tube, avoiding the pellet.
8. Store extracted and DNased RNA at –80°C.