RNeasy® Mini Kit Protocol

As modified for use with dolphin skin tissues on an Omni BeadRuptor

Notes before starting:

* If purifying RNA from cell lines rich in RNases, or tissue, add 10 μl

β-mercaptoethanol (β-ME) to 1 ml Buffer RLT. Buffer RLT with β-ME or DTT can be stored at room temperature for up to 1 month.

* Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
* Remove RNAlater®-stabilized tissue from the reagent using forceps.
* Keep RNAlater samples on ice, pre-clean all surfaces and materials with RNase Away. Prepare sufficient forceps, weigh boats, and razors for each sample to avoid cleaning between steps.
* Pre-label tubes ahead of time to reduce time between steps during protocol (at least for the steps before adding the sample to the column).
* Pre-fill Omni homogenization tubes with 600 uL of RLT buffer with β-mercaptoethanol and ceramic beads.
* Prepare a sufficient amount of fresh 70% ethanol prior to beginning.

1. For tissue homogenization:
   1. Cut a10-20 mg piece of tissue in a cleaned weight boat with a razor and place in Omni tubes with β-mercaptoethanol. Keep on ice (note: I don’t think this is necessary but I did it as a precaution).
   2. Place in Omni BeadRuptor making sure to properly balance the samples (at least 4 must be at the spots marked by arrows)
   3. Run once at 5 m/s for 45sec or 1 min (optimize this for the tissue type, size).
   4. Place samples on ice for 1-2 min (until cool)
   5. If necessary: place back in the Omni for 1 cycle. Remove, cool on ice briefly, and proceed to the next step.
2. Transfer the lysate to a lo-binding, RNase-free 1.5 mL tube. Make sure tissue is entirely homogenized. If not, place in homogenizer for one more cycle.
3. Centrifuge the lysate for 3 min at maximum speed (ran at approximately ≥15,000 x g). Carefully remove the supernatant by pipetting (approximately 500 μl), place it in a new RNase-free 1.5 mL tube, and proceed immediately to step 2.
4. Add 1 volume (500 μl) of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 5.
5. Transfer 500 μl (or up to 700 μl of the sample), including any precipitate, to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
6. Repeat step 5 with any remaining sample.
7. Add 700 μl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
8. Add 500 μl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
9. Add 500 μl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g.
10. Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 30 seconds to dry the membrane. (Optional, 1 min in the original protocol but can spin up to 5 min if necessary)
11. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 50 μl RNase-free water (30-50 recommended by the protocol) directly to the spin column membrane. Let sit at room temperature for 2 minutes (not necessary)
12. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.
13. Repeat step 7 using another 50 μl of RNase-free water. (or using the eluate from step 7 if high RNA concentration is required). Reuse the collection tube from step 7.
14. Immediately store at -80°C.

**Table 1. Volumes of Buffer RLT for sample disruption and**

**homogenization**

