

## **Disruption and Homogenization of Tissue for the Extraction of RNA Animal Tissue Protocol**

### **Tips for optimal tissue disruption and homogenization:**

- a. Use a tube for the homogenization that does not have a conical-shaped bottom. A round or flat bottom tube will allow for a better flow of the material through the probe.
- b. Place the tip of the probe half the distance from the bottom of the tube and against the side of the tube. This will minimize foaming
- c. When homogenization is complete, decrease the speed of the probe to low and gently tap the probe against the side of the tube and remove from the solution in order to minimize the amount of sample remaining in the probe.
- d. In the lab, we use the Omni Polytron ([www.omni-inc.com](http://www.omni-inc.com)) with disposable probes for most tissues. This is a hand held unit, which works nicely for disrupting tissue. The disposable probes remove the risk of sample cross contamination and reduce the time required to disrupt multiple samples since cleaning the probe is not required between samples. The probes are expensive but they can be cleaned and sterilized between uses. If you should notice any type of crack in the disposable unit, discard and use a new probe.
- e. The rotor-stator generator probe of choice for tissues with a high content of connective or fibrous tissue (i.e. skin, muscle etc.) is a saw tooth with oversized windows. The oversized windows allow for better flow of the tissue through the probe, while the saw tooth edge will quickly shear the tissue allowing for efficient and complete disruption of the tissue in a timely manner.
- f. If using disposable rotor-stator generator probes, only the probe needs to be changed between samples. For a steel rotor-stator generator probe, the probe should be disassembled, cleaned and autoclaved before use. Between samples, rinse in sterile water, followed by 3 washes in 70% ethanol and a final rinse in sterile water. Wipe with a Kimwipe™ after each wash.

### **Disruption and Homogenization of Frozen Tissue:**

1. A cube of tissue is quickly removed from the cryovial and weighed. The weight of the sample will determine which Qiagen RNeasy™ kit is to be used for the extraction. (<http://www1.qiagen.com/Products/RnaStabilizationPurification/OtherSamples.aspx>)
2. The weighed tissue is placed in a separate cyrovial and placed on dry ice until all samples have been weighed and are ready to disrupt.
3. Under a hood, prepare the lysis buffer by adding 10 ul of beta-mercaptoethanol ( $\beta$ ME) per 1 ml of RLT buffer. Make sure to prepare enough  $\beta$ /ME for all samples to be lysed. See the respective Qiagen RNeasy™ kit manual for the appropriate volume to add to each sample (<http://www1.qiagen.com/Products/RnaStabilizationPurification/OtherSamples.aspx>).
4. For the first sample, pour the tissue into a medium-sized weigh boat pre-filled with  $\beta$ ME/RLT buffer. Using 2 razor blades, mince the tissue. For optimal disruption of the tissue, no piece should be larger than half the diameter of the probe.

5. Pour the minced sample into a tube containing the remaining  $\beta$ ME/RLT buffer.
6. Homogenize the tissue at 15-20 second intervals resting for 5 seconds between each interval for a total of 60 seconds. At the intervals, the speed of the polytron is decreased and the probe gently tapped on the side of the tube. During the homogenization, the speed does not need to be on high to ensure complete disruption and lysis of the tissue. Half the speed will sufficiently disrupt the tissue without producing foam.
7. Proceed to the next sample.

**Disruption and Homogenization of Tissue Stored in RNAlater™:**

*When using RNAlater™, tissue does not have to be handled as rapidly as frozen tissue since the reagent is a preservative of the RNA.*

1. A cube of tissue is removed from the cryovial containing RNAlater™ and weighed. The weight of the sample will determine which Qiagen RNeasy kit is to be used for the extraction. (<http://www1.qiagen.com/Products/RnaStabilizationPurification/OtherSamples.aspx>)
2. The weighed tissue is placed in a separate cryovial containing .5 – 1.0 ml of RNAlater and placed on wet ice until all samples have been weighed and are ready to disrupt.
3. Under a hood, prepare the lysis buffer by adding 10  $\mu$ l of beta-mercaptoethanol ( $\beta$ ME) per 1 ml of RLT buffer. Make sure to prepare enough  $\beta$ /ME for all samples to be lysed. See the respective Qiagen RNeasy™ kit manual for the appropriate volume to add to each sample (<http://www1.qiagen.com/Products/RnaStabilizationPurification/OtherSamples.aspx>).
4. For the first sample, pour the tissue into a small weigh boat. Pipet off excess RNAlater™. Add the  $\beta$ ME/RLT buffer and mince the tissue using 2 razor blades. For optimal disruption of the tissue, no piece should be larger than half the diameter of the probe.
5. Pour the minced sample into a tube containing the remaining  $\beta$ ME/RLT buffer.
6. Homogenize the tissue at 15-20 second intervals resting for 5 seconds between each interval for a total of 60 seconds. At the intervals, the speed of the polytron is decreased and the probe gently tapped on the side of the tube. During the homogenization, the speed does not need to be on high to ensure complete disruption and lysis of the tissue. Half the speed will sufficiently disrupt the tissue without producing foam.
7. Proceed with the next sample.

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