User-Developed Protocol:

Purification of total RNA from fatty tissues using QIAzol Lysis Reagent, MaXtract High Density, and the TissueLyser

This protocol has been adapted by customers for the purification of total RNA from fatty tissues using QIAzol Lysis Reagent, MaXtract High Density, and the TissueLyser. This protocol can also be used with all other types of tissues. **This protocol has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please be sure to read the *QIAzol Handbook* and the *MaXtract Low and High Density Handbook* before starting, paying careful attention to the Safety Information section.

Equipment and reagents to be supplied by user

- QIAzol Lysis Reagent (200 ml) (cat. no. 79306)
- MaXtract High Density (200 x 2 ml) (cat. no. 129056)
- TissueLyser (to order, visit www.qiagen.com or contact your local QIAGEN office)
- Also refer to “Equipment and Reagents to Be Supplied by User” in the *QIAzol Handbook*

Important points before starting

- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions and *TissueLyser Handbook*. For other bead mills, refer to suppliers’ guidelines.
- If using QIAzol Lysis Reagent for the first time, read “Important Notes” in the *QIAzol Handbook* (October 2006 edition).
- Fresh, frozen, or RNA*later*® stabilized tissues can be used.* If freezing tissues, flash-freeze in liquid nitrogen and immediately transfer to –70ºC, where they can be stored for several months. Do not allow tissues to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates from step 3 can also be stored at –70ºC for at least 1 month. Incubate frozen lysates at 37ºC in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- In the procedure below, ▲ refers to use of the TissueLyser Adapter Set 2 x 24 with 5 mm diameter stainless steel beads (for <100 mg tissue), and ● refers to use of the TissueLyser Adapter Set 2 x 96 with 5 mm diameter stainless steel beads (for <75 mg tissue).

* RNA*later* RNA Stabilization Reagent cannot be used with adipose tissue due to the high abundance of fat, but can be used with other fatty tissues such as brain.

Procedure

1. **Add ▲ one stainless steel bead (5 mm mean diameter) per 2 ml microcentrifuge tube, or ● one stainless steel bead (5 mm mean diameter) per collection microtube. Place the tubes on dry ice.**

   The tubes do not need to be placed on dry ice if the tissue samples are stabilized in RNA*later* RNA Stabilization Reagent.

2. **Excise the tissue samples from the animal or remove them from storage. Determine the amount of each tissue. Place each tissue into a tube from step 1.**

   Weighing tissue is the most accurate way to determine the amount.

   If the tissue samples were stored in RNA*later* RNA Stabilization Reagent, remove them from the reagent using forceps and be sure to remove any crystals that may have formed.

   RNA in harvested tissues is not protected until the tissues are treated with RNA*later* RNA Stabilization Reagent, flash-frozen, or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. **Remove the tubes from the dry ice. Add QIAzol Lysis Reagent to each tube: 1 ml QIAzol Lysis Reagent per 100 mg tissue is required. The volume of tissue should not exceed 10% of the volume of QIAzol Lysis Reagent.**

   ▲ Place the tubes in the TissueLyser Adapter Set 2 x 24. Operate the TissueLyser for 2 min at 20 Hz. Then rearrange the tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser for another 2 min at 20 Hz.

   ● Close the collection microtubes using the collection microtube caps. Place the rack of tubes in the TissueLyser Adapter Set 2 x 96. Operate the TissueLyser for 2 min at 20 Hz. Then rotate the rack of tubes. Operate the TissueLyser for another 2 min at 20 Hz.

   The time and frequency depend on the tissue being processed and can be increased until the tissue is completely homogenized (e.g., up to 2 x 5 min at 25 Hz). Do not use frequencies higher than 25 Hz, since they can cause RNA degradation. Rearranging the tubes allows even homogenization. Do not reuse the stainless steel beads.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the TissueLyser or TissueRuptor generally results in higher RNA yields than with other methods.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x *g* for 10 min at 4ºC to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 4.

4. **Place the tubes containing the homogenates on the benchtop at room temperature (15–25ºC) for 5 min.**

   This step promotes dissociation of nucleoprotein complexes.

   ● Centrifuge the rack of collection microtubes at 6000 x *g* for 1 min at 15–25ºC to collect residual liquid from the caps of the tubes.

5. **Centrifuge 2 ml tubes of MaXtract High Density at 12,000–16,000 x *g* for 20–30 s. Transfer each homogenate to a MaXtract tube.**

   Note: If the homogenate was derived from RNA*later* stabilized tissue, add 50–100 µl RNase-free water to the MaXtract tube as well.

6. **To each MaXtract tube, add 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent pipetted in step 3. Securely cap the tubes, and shake vigorously for 15 s. Do not vortex.**

   Thorough mixing is important for subsequent phase separation.

7. **Place the MaXtract tubes on the benchtop at room temperature for 2–3 min.**

8. **Centrifuge at ▲ 12,000 x *g* or ● 6000 x *g* for 15 min at 4ºC.**

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase (MaXtract High Density gel); and a lower, red, organic phase. For tissues with an especially high fat content, an additional clear phase may be visible below the red, organic phase. The volume of the aqueous phase is approximately 60% of the volume of the QIAzol Lysis Reagent pipetted in step 3.

9. **Transfer the upper, aqueous phase to new tubes. Add 0.5 ml isopropanol per 1 ml QIAzol Lysis Reagent pipetted in step 3. Mix thoroughly by vortexing.**

10. **Place the tubes on the benchtop at room temperature for 10 min.**

11. **Centrifuge at 12,000 x *g* for 10 min at 4ºC.**

12. **Carefully aspirate and discard the supernatants.**

    The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.

13. **Add at least 1 ml of 75% ethanol per 1 ml QIAzol Lysis Reagent pipetted in step 3. Centrifuge at 7500 x *g* for 5 min at 4ºC.**

    If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x *g* for 5 min at 4ºC.

14. **Remove the supernatants completely, and briefly air-dry the RNA pellets.**

    Do not dry the RNA using a vacuum.

15. **Redissolve the RNA in an appropriate volume of RNase-free water. Clean up the RNA using the RNeasy® MinElute® Cleanup Kit or RNeasy Mini, Midi, or Maxi Kit.**

    We recommend RNA cleanup to remove contaminating phenol. The RNeasy MinElute Cleanup Kit and RNeasy Mini, Midi, and Maxi Kits allow cleanup of up to 45 µg, 100 µg, 1 mg, and 6 mg total RNA, respectively. For details, refer to the RNA cleanup protocol in the handbook supplied with these kits.

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