Isol-RNA Lysis Reagent and Phase Lock Gel

Purification of total RNA from fatty tissues using Isol-RNA Lysis Reagent and Phase Lock Gel (PLG)

This protocol has been adapted by customers for the purification of total RNA from tissue and especially fatty tissues using Isol-RNA Lysis Reagent and Phase Lock Gel. This protocol has not been thoroughly tested and optimized by 5 PRIME.

Note: Please be sure to read the Isol-RNA Lysis Reagent Handbook and the Phase Lock Gel Handbook before starting.

Equipment and reagents to be supplied by user

1. Isol-RNA Lysis Reagent (200 ml) (Ref. No. 2302700)
2. Phase Lock Gel Heavy (200 x 2 ml tubes) (Ref. No. 2302830)
3. Rotor-stator (e.g. TissueRuptor (QIAGEN))

Important Points before starting

1. Ensure that you are familiar with operating the Rotor-stator and refer to suppliers' guidelines.
2. If using Isol-RNA Lysis Reagent for the first time, read the Isol-RNA Lysis Reagent Manual.
3. Fresh, frozen, or stabilized tissues can be used. For long-term storage (up to several month), flash-freeze in liquid nitrogen and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Isol-RNA Lysis Reagent. Homogenized tissue lysates from step 4 can also be stored at –70°C for at least 1 month. Incubate frozen lysates at 37°C in a water bath only until the sample is completely thawed and salts are dissolved before continuing with step 5. Avoid prolonged incubation, which may compromise RNA integrity.
4. Isol-RNA Lysis Reagent contains a guanidine salt and is not compatible with disinfecting reagents containing bleach.
5. Except for phase separation, all protocol and centrifugation steps should be performed at room temperature (15–25°C). Work quickly during the procedure.

Procedure

1. Add Isol-RNA Lysis Reagent to an appropriate vessel (1 ml Isol-RNA Lysis Reagent per 100 mg tissue is required)

   This vessel will be used for disruption, homogenization and subsequent centrifugation.

   Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

   The volume of tissue should not exceed 10% of the volume of Isol-RNA Lysis Reagent.

2. Remove the sample from storage or excise from the donor.

   RNA in harvested tissues is not protected until the tissues are flash-frozen, treated with RNA*later* RNA stabilization Reagent (QIAGEN), or disrupted and homogenized. Frozen tissues should not be allowed to thaw during handling. Work as quickly as possible.

3. Determine the amount of tissue and place it into the Isol-RNA Lysis Reagent. Proceed immediately to the next step.

   Weighing tissue is the most accurate way to determine the amount.
   If the tissue sample was stored in RNA*later* RNA Stabilization Reagent, remove it from the reagent using forceps and be sure to remove any crystals that may have formed.

4. Place the tip of the rotor–stator homogenizer disposable probe into the Isol-RNA Lysis Reagent and operate the rotor–stator homogenizer at full speed until the tissue lysate is uniformly homogeneous (usually 20–40 s).

   Note: To avoid damage to the rotor–stator homogenizer and disposable probe during operation, make sure the tip of the probe remains submerged in the Isol-RNA Lysis Reagent.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the rotor–stator homogenizer or bead-mill 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 4°C to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 5.

5. Place the tube containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
   This step promotes dissociation of nucleoprotein complexes.

6. Centrifuge a 2 ml tube of Phase Lock Gel (PGL) Heavy at 12,000–16,000 x g for 20–30 s. Transfer the homogenate to the PLG tube.

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

7. To the PLG-tube, add 0.2 ml chloroform per 1 ml Isol-RNA Lysis Reagent pipetted in step 1. Securely cap the tube, and shake it vigorously for 15 s. **Do not vortex!**

   Thorough mixing is important for subsequent phase separation.

8. Place the PLG-tube on the benchtop at room temperature for 2–3 min.

9. Centrifuge at 12,000 x g for 15 min at 4°C.

   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase (Phase Lock Gel Heavy gel); and a lower, organic phase. For tissues with an especially high fat content, an additional clear phase may be visible below the organic phase. The volume of the aqueous phase is approximately 60% of the volume of the Isol-RNA Lysis Reagent pipetted in step 1.

10. Transfer the upper, aqueous phase to a new tube.

11. Add 0.5 ml Isopropanol per 1 ml Isol-RNA Lysis Reagent. Mix thoroughly by vortexing.

12. Place the tube on the benchtop at room temperature for 10 min.

13. Centrifuge at 12,000 x g for 10 min at 4°C.

14. Carefully aspirate and discard the supernatant.

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

15. Add at least 1 ml of 75% Ethanol per 1 ml Isol-RNA Lysis Reagent pipetted in step 1.

16. Centrifuge at 7,500 x g for 5 min at 4°C.

    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 4°C.

17. Remove the supernatant completely, and briefly air-dry the RNA pellet.

    Do not dry the RNA using a vacuum.

18. Redissolve the RNA in an appropriate volume of RNase-free water.