

Whole Mouse Skin

RNA ISOLATION USING TRIZOL REAGENT FOR TOTAL RNA and QIAGEN Mini-Column Clean-Up

Skin Preparation for Homogenization:

Work as quickly as possible until tissue is frozen.

Option 1

Sacrifice mouse; immediately shave. Cut out skin from the back (~4cm x 4cm, or 1 1/2" x 1 1/2"). Place on a clean weigh boat and quickly weigh (~250-500 mg for above size).

Wrap in foil and snap freeze in liquid nitrogen. Store at -70°C.

When ready to process, do one at a time until ground up in TRIzol (prior to adding chloroform) and keep tubes on ice. Keep unpulverized samples on dry ice.

Option 2

Sacrifice mouse; immediately shave. Cut out skin from the back (~4cm x 4cm, or 1 1/2" x 1 1/2").

Remove a small piece for histology. Quickly place remaining section on a clean weigh boat and weigh.

Transfer immediately to the waiting liquid nitrogen in the mortar, and proceed with the following steps.

Total RNA Extraction:

Volumes are based on skin sizes and weights mentioned above.

1. Place whole mouse skin into a mortar, filled with liquid nitrogen, that is sitting in a bucket of dry ice.
2. Grind the skin with a cold pestle, allowing the liquid nitrogen to sublime without adding more. Keep grinding until the skin is a powder-form.
3. Scrape powder into a pre-chilled tube (on dry ice) and keep in dry ice, or store at -70°C, until you are ready to extract with TRIzol (Gibco BRL).

Tissue Homogenization

Keep homogenization tubes containing measured TRIzol on wet ice. Clean homogenizer before use, between samples and after use with 0.1M NaOH then 1M Tris, then autoclaved, Rnase-free water.

Homogenize well with tube submerged in a beaker of wet ice.

1. Add 1 ml of TRIzol Reagent per 50-100 mg of initial tissue weight to homogenization tubes that are kept cold by sitting in wet ice.
2. Transfer frozen, powder-form skin to waiting TRIzol Reagent; vortex vigorously
3. Homogenize thoroughly (until it is one homogeneous solution). Foaming of the sample should be avoided. Proceed to next step.

Phase Separation

1. Incubate homogenized samples for 5 minutes, RT.
2. Add 0.3 ml chloroform (Molecular Biology grade, Sigma C2432) per 1 ml of TRIzol Reagent originally used.
3. Mix vigorously by hand for about 30 seconds; incubate, RT, for 3 minutes.
4. Centrifuge samples at 12,000 x g (~10,000 rpm), 15 minutes, 4°C.
5. Pipette off the top aqueous phase (RNA) – avoid protein interface, it will be big - and transfer to a new 13 ml homogenization / centrifuge tube. This will be ~60% of the volume of TRIzol used.
6. Add 1:1 (extracted aqueous phase to isopropyl alcohol. Cap and invert 10x to mix.
7. Incubate for 10 minutes, RT.
8. Centrifuge samples at 12,000 x g (~10,000 rpm), 10 minutes, 4°C.
9. RNA precipitate forms a gel-like pellet on the side and bottom of the tube.
10. Pour off the supernatant.
11. Wash the RNA pellet once with 600 µl of 75% RNase-free prepared ethanol (Sigma E702-3). Centrifuge <7500 x g (~8500 rpm), 5 minutes, 4°C.
12. Gently pour off ethanol and place tube upside down on a clean paper and allow to air-dry for 15 minutes.

13. Resuspend pellet in (50, 100, or 200 μ l) RNase-free water depending on the size of the pellet. Heat 10 minutes, 60°C, to facilitate resuspension.
Once dissolved, always keep RNA on ice and store at -70°C.

Quantitation of total RNA

The concentration of RNA should be determined by measuring the absorbance at 260nm in a spectrophotometer. Expected yield from 4 x 4 cm size of skin (250–500 mg) is 250-500 μ g total RNA (~1 μ g/1mg).

Purity of RNA

The ratio of the readings at 260nm and 280nm provides an estimate of the purity of RNA. A 260/280 ratio of 1.6 to 1.7 is good for RNA in water (it should be higher if in buffer).

Integrity of RNA

The integrity and size distribution of total RNA should be checked by denaturing agarose gel electrophoresis and ethidium bromide staining. Take a photograph of the gel to submit with your samples.

Qiagen Clean-Up:

Refer to the Qiagen Mini-column handbook for details of this protocol.

Yield of RNA is improved by using multiple mini-columns (instead of 1 or 2 midi-columns) and combining eluates at the end.

Quantitate and store at -70°C.

Concentration and Storage of RNA for NIEHS Microarray Group

Concentrate with a pre-wetted (with 20 μ l RNase-free water) Microcon-30 filter unit (Amicon #42410) to the defined concentration by spinning at 12,000 x g (~10,000 rpm), RT. Total spin time will vary according to how much starting sample you have and % reduction desired. When at a desired volume, invert column unit over a clean tube and spin 2 minutes at 12,000 x g (~10,000 rpm), RT.

Recheck the concentration and store at -70°C.

For NIEHS In-House Produced Arrays:

Aliquots of 100 μ g should be stored at 2 μ g/ μ l in RNase-free water.

For Agilent Commercial Arrays:

Aliquots of 60 μ g should be stored at 1.1 – 5.0 μ g/ μ l in RNase-free water.