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# Liver Tissue RNA Isolation - University of Minnesota TMCs

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

InvitrogenTM TRIzolTM Plus RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of samples, including animal and plant cells and tissue, bacteria, and yeast.

This protocol was used to isolate RNA from liver tissue for BulkRNA sequencing in collaboration with The University of Minnesota Genomics Center.

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## Isolate RNA

1 a. Lyse and homogenize samples in TRIzol Reagent by adding 1mL TRIzol reagent per 50-100mg tissue to the sample and use a homogenizer
b. Incubate for 5 minutes to permit complete dissociate of the nucleoproteins complex
c. Add 0.2mL of chloroform per 1mL TRIzol Reagent used for lysis, then securely cap the tube.
d. Incubate for 2-3 minutes.
e. Centrifuge the sample for 15 minutes at 12000 x g at 4 °C - the mixture separates into a lower red phenol-chloroform, interphase and a colorless upper aqueous phase.
f. Transfer ~600 µL of the colorless upper phase containing the RNA to a new tube
g. Add an equal volume of 70% ethanol, then mix well by vortexing.
h. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

## Bind the RNA to the membrane

2 a. Transfer up to 700 µL of the sample to a spin cartridge with (collection tube).
b. Centrifuge 12000 x g for 15 seconds.
c. Discard the flow through, then reinsert the spin cartridge into the same collection tube.
d. Repeat step 2a -step 2c until the entire sample has been processed.

## Wash the RNA on the membrane

3 a. Add 700 µL of Wash Buffer I to the spin cartridge.
b. Centrifuge at 12000 x g for 15 seconds.
c. Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
d. Add 500 µL of Wash Buffer II to the spin cartridge.
e. Centrifuge at 12000 x g for 15 seconds.
f. Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
g. Repeat step 3d-3f once.

## Elute the RNA

4 a. Centrifuge at 12000 x g for 1 minute to dry the membrane.
b. Discard the collection tube, then insert the spin cartridge into a recovery tube.
c. Add 30 µL-3 × 100 µL (3 sequential elutions with 100 µL each) of RNase-free water to the center of the spin cartridge.
- Note: If you are performing sequential elutions, collect all eluates in the same tube.
d. Incubate for 1 minute.

e. Centrifuge at >12,000 × g for 2 minutes.
f. Discard the spin cartridge.
The recovery tube contains the purified total RNA.

- Store the purified RNA on ice if used within a few hours. For long-term storage, store the purified RNA
  at −80°C.
- If highly pure RNA without genomic DNA contamination is required, perform DNase I treatment after
  purification (see PureLinkTM RNA Mini Kit User Guide (Pub. No. MAN0000406).