RNA extraction with trizol for tissues

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Abstract

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Before start

Clean the benches and all the material that will be used with alcohol 70.

Use tips with filter.

Cool the centrifuge to 4°C.

Materials

- EDTA by Contributed by users
- 75% Ethanol by Contributed by users
- Isopropanol by Contributed by users
- Chloroform by Contributed by users
- RNase-free water by Contributed by users

Protocol

RNA extraction

Step 1.

Add 1 mL of Trizol to amounts of 50 to 100 mg and homogenize with the homogenizer. Pass to a tube.

In this step, you can freeze this sample for up to 6 months in the -80C freezer or continue the protocol.

RNA extraction

Step 2.

Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

RNA extraction

Step 3.

Add 200 µl L of chloroform per 1 mL of TRIzol and incubate for 2 to 3 minutes.

RNA extraction

Step 4.

Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.

RNA extraction

Step 5.

The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.

Transfer the aqueous (incolor) phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

RNA extraction

Step 6.

Add 500 µl of isopropanol per 1 mL of TRIzol. Incubate for 10 minutes.

RNA extraction

Step 7.

Centrifuge for 10 minutes at 12.000 × g at 4°C.

RNA extraction

Step 8.

Discard the supernatant with a micropipetto and resuspend the pellet in 1 mL of 75% ethanol per 1 mL of TRIzol.

RNA extraction

Step 9.

Vortex the sample briefly and centrifuge for 5 minutes at 7.500 × g at 4°C.

RNA extraction

Step 10.

Discard the supernatant with a micropipettor. Vacuum or air dry the RNA pellet for 5–10 minutes.

RNA extraction

Step 11.

Resuspend the pellet in 20–50 µL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.

Note: Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.

RNA extraction

Step 12.

Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.

Then store in -80°C freezer.

Step 13.