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 We use this protocol and it's working

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RNA extraction with trizol for tissues

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MATERIALS

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EDTA Contributed by users

75% Ethanol Contributed by users

Isopropanol Contributed by users

Chloroform Contributed by users

RNase-free water Contributed by users

BEFORE START INSTRUCTIONS

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

Use tips with filter.

Cool the centrifuge to 4C.

RNA extraction

- 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

- 2 Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
- 3 Add 200 µl L of chloroform per 1 mL of TRIzol and incubate for 2 to 3 minutes.
- 4 Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.
- 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.
- 6 Add 500 µl of isopropanol per 1 mL of TRIzol. Incubate for 10 minutes.
- 7 Centrifuge for 10 minutes at 12.000 × g at 4°C.
- 8 Discard the supernatant with a micropipetto and resuspend the pellet in 1 mL of 75% ethanol per

1 mL of TRIzol.

9 Vortex the sample briefly and centrifuge for 5 minutes at $7.500 \times g$ at 4°C .

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

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.

12 Incubate in a water bath or heat block set at $55\text{--}60^{\circ}\text{C}$ for 10–15 minutes.

Then store in -80°C freezer.

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