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Version 1

Extraction RNA of cells by Trizol V.1

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Protocol status: Working

We use this protocol and it's working

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Materials

MATERIALS

- ⊗ RNAse-free Water
- ⊗ TRIzol Reagent **Thermo Fisher Scientific Catalog #15596026**
- ⊗ Isopropanol
- ⊗ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988**
- ⊗ 0.5% Sodium dodecyl sulfate solution
- ⊗ Ethanol 75%
- ⊗ 0.1 mM EDTA - Ethylenediaminetetraacetic acid

Troubleshooting

Safety warnings

- ! The triazole reagent is toxic, gloves, lab coat, mask should be used. Manipulate the trizol in the exhaust hood.

Before start

Clean the work area with 70% alcohol. Use all filters and autoclaved tips. Refrigerate your centrifuge to 4C.

- 1 Remove the culture medium from the plate and add 750µl of Trizol to 1×10^5 - 10^7 cells. Homogenize with the pipette and transfer to a tube.
In this step, you can freeze this sample for up to 6 months in the -80C freezer or continue the protocol.
- 2 Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
- 3 Add 200 µl of chloroform per 1 mL of Trizol and incubate for 2 to 3 minutes.
- 4 Centrifuge the sample for 15 minutes at 12,000 xg at 4°C.
The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
- 5 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 7500 × 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.
- 12 Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.
- 13 Store in freezer -80°C until use or make the cDNA reaction then.

