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RNA Extraction with Trizol for cells V.2

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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

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.
750 µL Trizol
-80 °C freezer

2 Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
00:05:00 Incubation

3 Add 200 µl L of chloroform per 1 mL of Trizol and incubate for 2 to 3 minutes.
00:02:00 Incubation

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.
4 °C Centrifugation
00:15:00 Centrifugation

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.
00:10:00 Incubation

7 Centrifuge for 10 minutes at 12,000 × g at 4°C.
4 °C Centrifugation
00:10:00 Centrifugation

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.
4 °C Centrifugation
00:05:00 Centrifugation

10 Discard the supernatant with a micropipettor. Vacuum or air dry the RNA pellet for 5–10 minutes.
00:05:00 Vacuum or air dry

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.

20 µL RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution

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

55 °C water bath

00:10:00 water bath

13 Store in freezer -80°C until use or make the cDNA reaction then.

-80 °C storage