RNA Extraction with Trizol for cells Version 2

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Abstract

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Before start

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

Materials

- RNAse-free Water by Contributed by users
- TRIzol Reagent 15596026 by Thermo Fisher Scientific
- Isopropanol by Contributed by users
- Chloroform 319988 by Sigma
- 0.5% Sodium dodecyl sulfate solution by Contributed by users
- Ethanol 75% by Contributed by users
- 0.1 mM EDTA - Ethylenediaminetetraacetic acid by Contributed by users

Protocol

Step 1.

Remove the culture medium from the plate and add 750µl of Trizol to 1 x 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.

AMOUNT
750 µl : Trizol
TEMPERATURE
-80 °C : freezer

Step 2.

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

DURATION
00:05:00 : Incubation

Step 3.

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

DURATION
00:02:00 : Incubation

Step 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.

TEMPERATURE
4 °C : Centrifugation
DURATION
00:15:00 : Centrifugation

Step 5.

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

Step 6.

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

DURATION
00:10:00 : Incubation

Step 7.

Centrifuge for 10 minutes at 12,000 × g at 4°C.

TEMPERATURE
4 °C : Centrifugation
DURATION
00:10:00 : Centrifugation

Step 8.

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

Step 9.

Vortex the sample briefly and centrifuge for 5 minutes at 7500 × g at 4°C.

TEMPERATURE
4 °C : Centrifugation
DURATION
00:05:00 : Centrifugation

Step 10.

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

DURATION
00:05:00 : Vacuum or air dry

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.

AMOUNT
20 µl : RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution

Step 12.

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

TEMPERATURE
55 °C : water bath
DURATION
00:10:00 : water bath

Step 13.

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

TEMPERATURE
-80 °C : storage

Warnings

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