VERSION 2

AUG 22, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ssheeb6

Protocol Citation: Maria Crmen Sales, Maryana Branquinho, Maysa Silva 2018. RNA Extraction with Trizol for cells . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.ssheeb6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Aug 21, 2018

Last Modified: Aug 22, 2018

PROTOCOL integer ID:
 14889

RNA Extraction with Trizol for cells V.2

Maria Crmen Sales¹, Maryana Branquinho¹, Maysa Silva¹

¹Universidade de So Paulo

Maria Crmen Sales
 Universidade de So Paulo

MATERIALS

MATERIALS

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

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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 750l of Trizol to 1 x 10⁵-10⁷ 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 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 micropipette 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