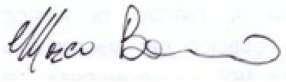
- Spin 100,000-500,000 cells at 1,200 rpm, discard the cell culture medium and wash the pellet (the optimal amount is 350,000 cells) with 5 ml of PBS (the pellet can be stored at -80 degrees or directed used). If a fresh cell pellet is used for RNA extraction, it has to be stored in ice until the protocol is started.
- Clean the work surface and the micropipettes with NaOH 10 mM to avoid RNAse activity.
- If for RNA extraction a frozen cell pellet is used, before to start with the protocol thaw the cell pellet for 10-15 minutes in ice, so proceed with the assay.
- If the following analysis does not require specific method for RNA extraction, the RNA extraction should be performed using TRIzol reagent.

TRIZOL PROTOCOL (Cells grown in suspension):

- Add 0.75 mL of TRIzol™ Reagent per 5–10 × 10^6 cells
- Pipet the lysate up and down several times to homogenize
- STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.
- Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
- Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.
- Incubate for 2–3 minutes.
- Centrifuge the sample for 15 minutes at 12,000 × g at 4°C (the mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase).
- Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45°.
- IMPORTANT! Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.
- Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of TRIzol™ Reagent used for lysis.
- Incubate for 10 minutes.
- Centrifuge for 10 minutes at 12,000 × g at 4°C.
- Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
- Discard the supernatant.
- Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of TRIzol™ Reagent used for lysis.
- STOPPING POINT The RNA can be stored in 75% ethanol for at least 1 year at –20°C, or at least 1 week at 4°C.
- Vortex the sample briefly then centrifuge for 5 minutes at 7500 × g at 4°C.
- Discard the supernatant
- Dry the RNA pellet for 5–10 minutes.
- 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.
- IMPORTANT! Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.
- Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.



- Proceed to downstream applications, or store the RNA at –70°C.

Advantages: possibility to isolate RNA/DNA and protein from the same sample.

Application: RNA/miRNA extraction.

Time: 1 hour.

Cost: <2 € per sample

TRIZOL PROTOCOL CAN BE FOUND AT:

https://tools.thermofisher.com/content/sfs/manuals/trizol_reagent.pdf

For the isolation of RNA with RNeasy Mini Kit please visit the website:

https://www.qiagen.com/us/resources/resourcedetail?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en

Briefly:

RNEASY MINI KIT PROTOCOL (Cells grown in suspension; do not use more than 1 x 10^7 cells):

- Pellet the cells by centrifuging for 5 min at 300 x g . Carefully remove all supernatant by aspiration.
- Disrupt the cells by adding Buffer RLT (350 ul for <5x10^6 cells; 600 ul for 5x10^6-1x10^7)
- Homogenize the lysate by pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe.
- Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.
- Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.
- Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.
- Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.
- Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane.

- Optional: Place the RNeasy spin column in a new 2 ml collection tube, and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.
- Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x g (≥10,000 rpm) to elute the RNA.

Advantages: possibility to isolate RNA from < 250,000 cells.

Time: 30 minutes.

Cost: 7 € per sample

For the isolation of RNA from very small sample (i.e. <50,000 cells) it is recommended to use the **RNeasy micro kit**:

https://www.qiagen.com/us/resources/download.aspx?id=682963a5-737a-46d2-bc9f-fa137b379ab5&lang=en

Cost: 10 € per sample.