1. TRIZOL® Reagent

The protocol should not be used for large numbers of cells (more than 50 Mio.) or more than 1 g of tissue. In this case, refer to “stepwise RNA preparation protocol!

1.1 Homogenization:

b. Cells grown in monolayer:

Wash cells with sterile ice-cold PBS. Lyse cells directly in a culture dish by adding 1 mL TRIzol reagent per 10 cm^2. *(The amount of Trizol reagent added is based on the area of the culture dish, at least →1mL / 10 cm^2. An insufficent amount of TRIzol reagent results in contamination with DNA and low yield.)*

1 ml	*3.5 cm*
3 ml	*6 cm*
8 ml	*10 cm*

Pass the cell lysate several times through a pipette and use polytron instantly. Never trypsinize cells for RNA prep.! If you need to collect cells, scrape them.

Optional:

An additional isolation step my be required for samples with high content of protein. Remove insoluble material by centrifugating at 12.000 x g for 10 min. at 4°C. The supernate contains the RNA. Transfer the cleared homogenate to a fresh tube. Continue with phase separation step.

1.2 Phase separation:

- **Incubate** the homogenized samples for **5 min at 15-30°C** to permit complete dissociation of nucleoprotein complexes.

- **Add 0,2 mL of chloroform** (per 1 mL TRIzol). Close lid. Shake tubes vigorously by hand for approx. 15 sec. and incubate at 15-30°C for 2-3 min.

- **Centrifuge** samples at 12.000 x g for 10 min. at 4°C → Phase separation appears.

  lower red Phase = phenol-chloroform (organic)

  interphase

colorless upper aqueous phase = RNA

- Carefully transfer all aqueous phase into fresh tube.

1.3 RNA precipitation:

- **Transfer** upper phase to a fresh tube (save organic phase if isolation of DNA / protein is desired).
- Mix with **Isopropanol: 0,5 mL** isopropanol (per 1 mL TRIzol). Incubate at RT for 10 min → centrifuge at 12.000 x g for 10 min at 4°C, remove supernatant.
- RNA precipitate forms a gel-like pellet on side and bottom.

1.4 RNA Wash: (~EtOH preequilibrated at 4°C)

- Remove supernatant
- Wash RNA pellet 1x with 75% EtOH (ice-cold), (at least 1 mL per 1mL TRIzol)
- Mix by vortexing
- centrifuge at no more than 7.500 x g for 2 min. 4°C.
- Air-dry the pellet

1.5 Redissolving the RNA:

Dissolve RNA in appropriate vol. (500 µl) of RNAse free water (sigma water) by passing a few times trough pipette.

Additional purification: Reextract at least 1 x with Ph/Chl: Add 1 µl of glycogen. Add 0.8 vol. of Phenol/Chloroform (200µl/200µl), vortex 10 sec. And spin 5 min at 12.000 x g 4°C. Transfer supernatant in fresh tube. If there's still interphase or dark colour, repeat this step.

Then, add 1 vol. of Chloroform (500µl), vortex and spin as above. Transfer supernatant in fresh tube. Add 0.1 vol. of 3 M NaAc (50µl) and 2 vol of abs EtOH (1000µl). Mix and spin 10 min at 12.000x g. Discard supernatant and wash RNA pellet with 0.5 ml of 70 % EtOH (ice-cold). Vortex, spin briefly and remove spnt completely. Air-dry. Resuspend in 100-500 µl of RNA grade water.

1.6 Photometry and storage:

Determine amount by **photometry** (use microcuvettes, Photometry in TE (1 OD_{260nm} = 40 µg/ml RNA; OD 260/280 should be more than 2) and quality by electrophoresis (Load 1, 2 and 5 µl).

For storage, add 0.1 vol of 3 M NaAc pH 5.5, 2 vol. EtOH abs., mix and keep at -80°C.
Never store RNA in water!

To remove a given amount of RNA for experiments: vortex briefly, remove desired amount of suspension and transfer in Eppendorf tube. If working with less than 10 µg, add 1 µl glycogen. Spin 10 min at 14.000 rpm at RT, remove spnt. Wash 1 x with 70 % EtOH, spin briefly, discard and air-dry RNA pellet.

Remarks:

1. Isolation of RNA from small quantities of tissue → < 1 to 10 mg or cells < 10^2 to 10^4 add 800 µl of TRIzol Reagent to the tissue or cells !
Add 20 µg glycogen with final concentration in TRIzol of 250 µg/mL. The glycogen remains in the upper aqueous phase.