RNA Extraction Protocol

This protocol provides a simple, reliable, and rapid method for isolating high-quality total RNA from cells and tissues. It is free of protein and DNA contamination. It can be used for Northern blot analysis, in vitro translation, RNase protection assay and molecular cloning.

- After separation of mononuclear cells and before place them at – 70 °C, add 1 ml **TRIzol Reagent** (Invitrogen).
- Thaw for complete dissociation of nucleoprotein complexes and homogenize the sample by repetitive pipetting up and down.
- Transfer it in a microcentrifuge tube.
- Add 200 µl **CIAA** (chloroform : isoamylic alcohol) 24:1.
- Shake the tube vigorously by hand for *10–15 sec* because vortexing may increase DNA contamination of your RNA sample. Incubate at room temperature for *2–3 min*.
- Centrifuge the sample at 13.000 for *15 min* at 4 °C.
- Transfer 500 µl of the colorless, upper phase containing the RNA to a fresh RNase-free tube.
- Add 500 µl of **Isopropyl alcohol**. Mix gently by hand and incubate at room temperature for *10 min*.
- Centrifuge at 13.000 rpm for *10 min* at 4 °C.
- Discard the supernatant. The RNA precipate, often invisible before centrifugation, forms a gel like pellet on the bottom of the tube.
- Wash the RNA by adding 1ml **70% ethanol**. Mix the sample by vortexing.
- Centrifuge at 8.000 rpm for *5 min* at 4 °C.
- Discard the supernatant.
- Briefly dry the RNA pellet at room temperature for *10 min*.
- Dissolve RNA by adding 15–40 µl (depending on the pellet) **RNase free H_2O** and passing the solution a few times through a pipette tip.
- Incubate for *10 min* at 60 °C.
- Run in a gel electrophoresis of agaroze 1.5% (1 µl loading dye and 2 µl RNA).
- Store the purified RNA on ice if used within a few hours. For long-term storage, store the purified RNA at –80°C.

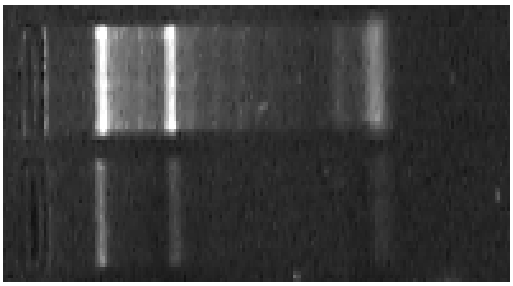
Notes :

1. Pre-warm the thermoblock at 60 °C (step 16).
2. Set the centrifuge at 4 °C.
3. After centrifugation (step 6), the mixture separates into a lower, red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 µl.
4. After homogenization and before addition of chloroform, samples can be stored at –60 to –70 for at least one month. The RNA precipitate can be stored in 70% ethanol at 2 to 8 °C for at least one week, or at least one year at –5 to –20 °C.
5. Both Isopropyl alcohol and Ethanol 70% should be at 4 °C.
6. Always use proper microbiological aseptic techniques when working with RNA. Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipette tips and tubes. Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material.

RNA extraction protocol from whole blood in EDTA tubes

- Shake the EDTA whole blood tube vigorously by hand.
- Transfer 1 ml of whole blood in 2 fresh tubes.
- Centrifuge at 3.500 rpm for 15 min at 24 °C (room temperature).
- Discard the supernatant and transfer the interphase in a fresh tube.
- Add 1 ml Trizol and mix vigorously by hand and by repetitive pippetting up and down.
- Incubate at –20 °C for 15-20 min.
- Shake vigorously the samples by hand.
- Transfer 1ml of the sample in fresh microcentrifuge tubes.

At this point continue the process as it is from the step 4 of the above protocol.



→ RNA template (2µl)

→ RNA sample (2µl)