Total RNA and Protein Extraction Kit (Cat. RP-01)

Description:

- This kit is designed for rapidly isolating the highest yields and quality of total RNA and protein from cells/tissue samples, offering a simple, fast, environmental-friendly protocol for total RNA/protein extractions without using vacuum filtration and toxic organic solvents such as xylene, phenol or chloroform. This kit is specially designed for preparing the high quality of RNA/protein from small amounts of sample material for RT-PCR, qPCR, microarrays, western blots and other RNA/protein assays. The kit is compatible with cells/tissues from LCM samples.
- **Key features:**
  1. Obtain the highest yield and integrity of total RNA and total protein within 1 hour.
  2. No filter column or vacuum filtration is required, able to avoid the loss of RNA during extracting.
  3. Suitable for extracting the pure total RNA/protein from the small/tiny tissues or LCM samples.

Kit components and protocol: (keep all samples and buffers on ice during proceeding)

Components:	Quantity (50 extractions)	Storage
One-step Fast Lysis Buffer-A contains nuclease/protease inhibitors	10.0 mL	-20°C
One-step I-Lysis Buffer-B contains nuclease/protease inhibitors	1.25 mL	-20°C
RNA Precipitation Solution (2x)	10.0 mL	-20°C
Protein Extraction Buffer-C	1.25 mL	-20°C

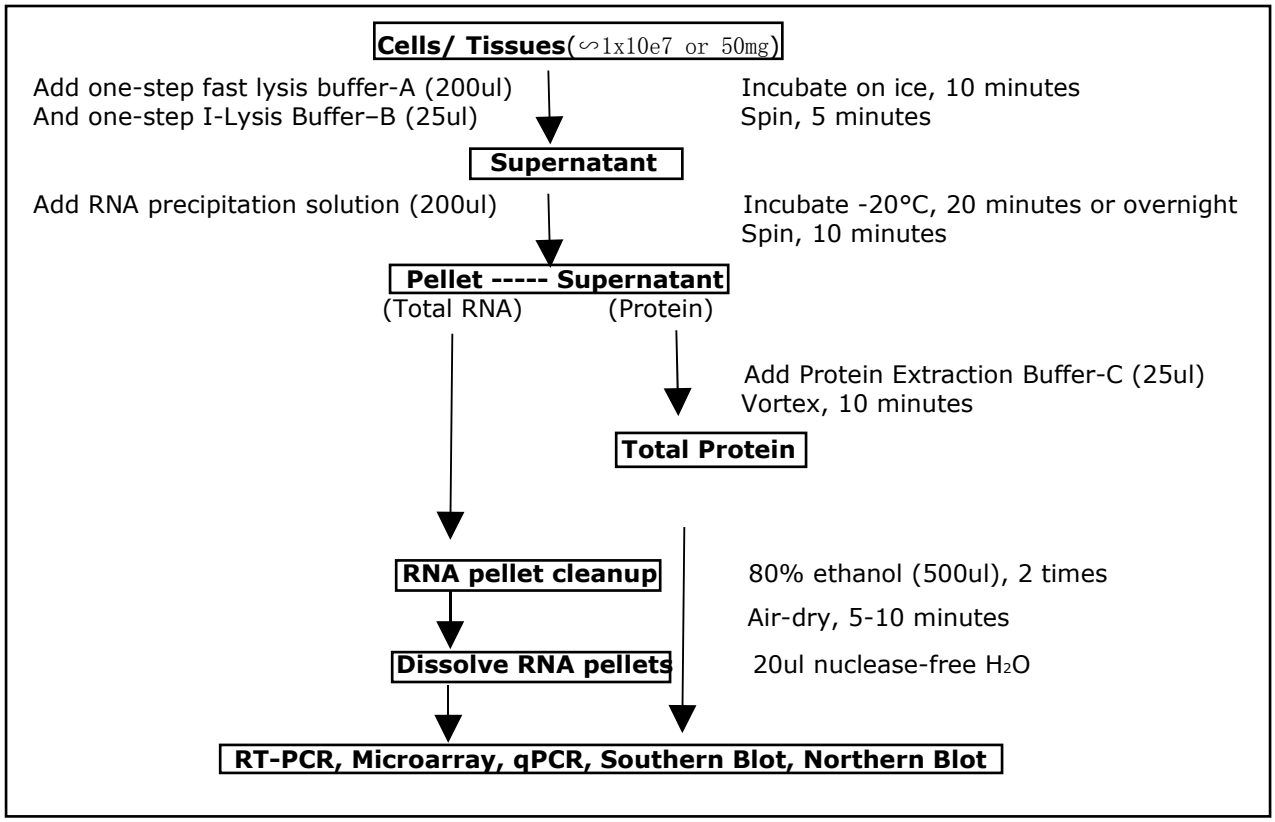
1. Add 200ul ice-cold **one-step fast lysis buffer-A** and 25ul **one-step I-Lysis buffer-B** into a clean 1.5ml mcrocentrifuge tube containing 50mg-200mg frozen/ fresh tissues or 1x10e7 cells. Incubate on ice for 15 minutes. Vortex vigorously at highest speed for 20 seconds every 5 minutes.
2. **(Optional)** Using a clean plastic pestle to homogenize the tissues for 10-20 strokes to completely disrupt the tissues and may obtain higher yields of total RNA and proteins.
3. Centrifuge at highest speed (∽13,000 xg) for 5 minutes at 4°C and transfer all the supernatant into a clean new 1.5mL tube. Discard the pellet.

   Note, Discard the lipoproteins/lipids that may form an upper layer after centrifugation.
4. Add 200ul **RNA Precipitation Solution (2x)** into the supernatant, mix thoroughly by pipette up and down several times.
5. Centrifuge at 5,000 xg for 2 minutes at 4°C and transfer the supernatant into a clean new 1.5mL tube and incubate at -20°C for 20 minutes or overnight. Save the pellet tube on ice and label as **total protein**.
6. Centrifuge the supernatant at highest speed (∽13,000 x g) for 10 minutes at 4°C. The pellet is the total RNA, and the supernatant contains protein fragments. Keep the pellet (RNA) on ice or continue the **step 8** for RNA cleanup.
7. Transfer the supernatant into the **total protein** tube from **step 5**, and add 25ul **protein extraction buffer-C,** incubate on ice for 10 minutes, vortex vigorously at highest speed for 20 seconds every 5 minutes. This suspension contained the total protein is ready for most protein assays: 1D and 2D electrophoresis and Western blotting.

   Note, centrifuge at highest speed (∽13,000 xg) for 2 minute at 4°C prior to measure the protein concentration using a spectrometer or BCA assays. Store the protein solution at -80°C.

8. Simply rinse the RNA pellet with 500ul ice cold 80% ethanol for 2 times without resuspending the RNA pellet. Centrifuge at highest speed (∽13,000 xg) for 10 minute at 4°C if the pellets are resuspended. Air-dry the RNA pellet for 5-10 minutes.
9. Dissolve the RNA pellets in 20ul nuclease-free H_2O or TE. Centrifuge at highest speed (∽13,000 xg) for 2 minute at 4°C prior to measure the RNA concentration with 260/280nm.
10. Store all the RNA/protein extracts at -80°C.

Flow Chart of RNA/protein Extraction: (an innovative RNA precipitation technology)



Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process. **Contact us,** Phone: 514-702 7702 Fax: 514-254 5356 Web:www.zmtechscience.com Email:order@zmtechscience.com