ZmTech® Total RNA, MicroRNA and Protein Extraction Kit (Cat. RMP-01)

Description:

This kit is designed for rapidly isolating the highest yields and quality of total RNA, microRNA and pure, native protein fragments from cells/tissue samples, offering a simple, fast, environmental-friendly protocol for total RNA/MicroRNA/protein extractions without using vacuum filtration and toxic organic solvents such as xylene, phenol or chloroform. This kit is specially designed for preparing the pure RNA/DNA/protein from small amounts of sample material for RT-PCR amplification, qPCR, micro-RNA assays, microarray, 1D/2D electrophoresis and western blotting. The kit is compatible with cells/tissues from LCM samples.

- **Key features:**

1. Obtain the highest yield and integrity of total RNA, Micro-RNA and pure, native proteins.
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/MircoRNA/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 extracts)	Storage
One-step Fast Lysis Buffer-A contained nuclease/protease inhibitors	10.0 mL	-20°C
One-step Neutralized Buffer-B	500 uL	-20°C
RNA Precipitation Solution (2x)	10.0 mL	-20°C
Protein Extraction Solution contains protease /phosphatase inhibitors	2.5 mL	-20°C
MicroRNA Precipitation Solution (2x)	20.0 mL	-20°C

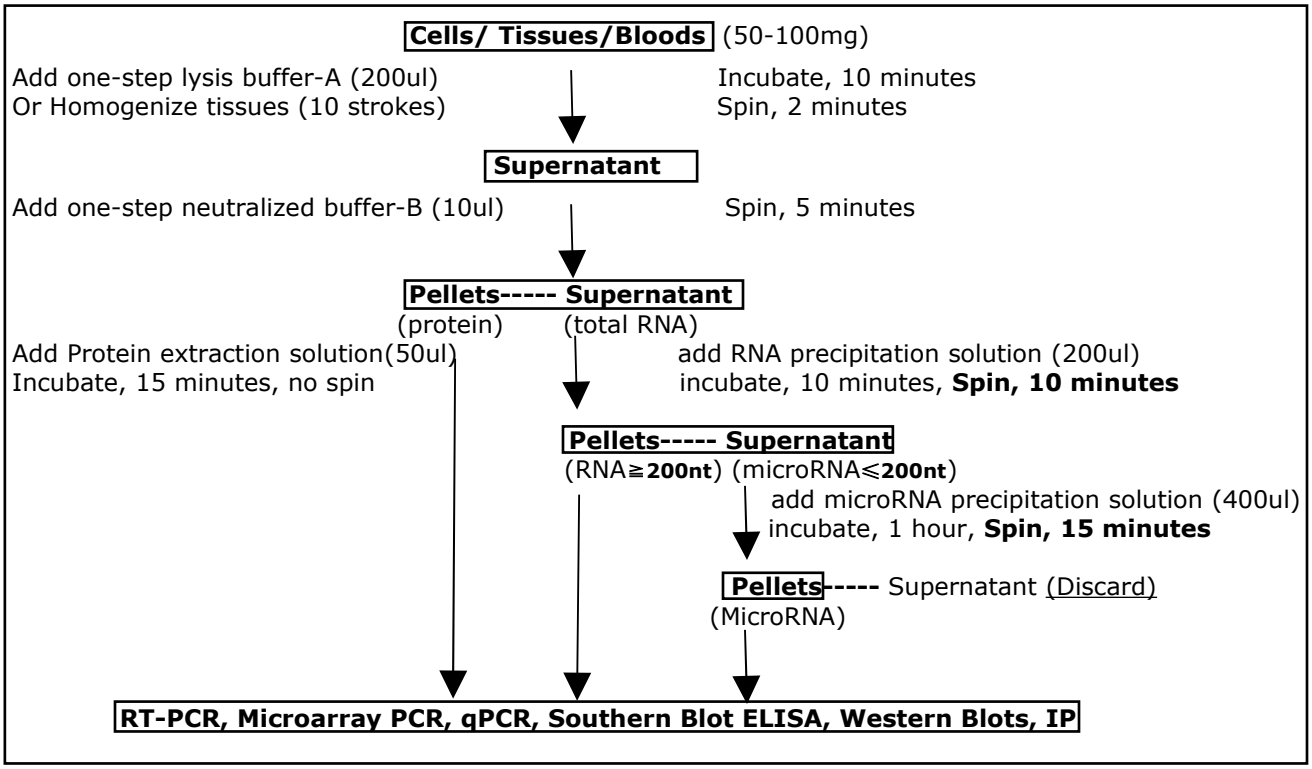
1. Add 200ul ice-cold **one-step fast lysis buffer-A** into a clean 1.5ml mcrocentrifuge tube containing 1-10x 10e6 cells or 50mg-200mg frozen/ fresh tissues and incubate on ice for 10-20 minutes. Vortex vigorously at highest speed for 20 seconds every 5 minutes.
2. **(Optional)** Using a clean plastic pestle to homogenize the tissues for 10-20 strokes may obtain higher yields of total RNA, microRNA and proteins.
3. Centrifuge at 1,000 xg (∽5500 rpm) for 2 minutes at 4°C and transfer the supernatant into a new clean 1.5ml tube. Discard the pellet (cell debris).
4. Add 10ul **one-step neutralized buffer-B** into the supernatant, mix thoroughly by pipette up and down several times.
5. Centrifuge at 13,000 xg for 5 minutes at 4°C and transfer the supernatant **(total RNA)** into a new clean 1.5ml tube. Save the pellet (**total protein**) tube on ice.
6. Resuspend the pellet **(total protein)** with 50ul **protein extraction solution** and incubate on ice for 15 minutes. Vortex vigorously at highest speed for 10 seconds every 5 minutes.
   Measure the protein concentration using a spectrometer. Store the total protein at -80°C.
7. Add 200ul **RNA Precipitation Solution (2x)** into the supernatant **(total RNA)**, mix thoroughly and incubate at -20°C for 10-20 minutes.
8. Centrifuge at highest speed (∽ 14,000 xg) for 10 minutes at 4°C. The pellet is the RNA (≧ 200nt) and the supernatant contains the MircoRNA (≤ 200nt). Transfer the supernatant **(MicroRNA)** into a new clean 1.5mL tube. Keep the pellet tube at -20°C.
9. Add 400ul **MicroRNA precipitation solution (2x)** into the supernatant (**MicroRNA**) tube. Mix thoroughly and incubate at -20°C for 1-2 hours or overnight. Centrifuge at highest speed (∽ 14,000 xg) for 15 minutes at 4°C. Carefully remove the supernatant. This pellet is the microRNA.

10. Rinse the RNA pellets from **Step8 (total RNA ≧200nt) and Step 9 (MicroRNA≤200nt)** with 500ul ice cold 80% ethanol for 3 times without resuspending the RNA pellet. The RNA pellets will not be visible if the concentration is less than 20ng/ul. Air-dry pellet for 5-10 minutes and dissolve the RNA pellets in 20ul nuclease-free H_2O or TE.
11. Measure the concentration of the MicroRNA **(≤200nt)** and the total RNA **(≧200nt)** with 260/280nm and store all the RNA extracts at -80°C.

Suggested Protocol for DNase I treatment prior to RT-PCR:

1. Mix 20ul RNA solution (≧ 10ug RNA) with 2ul of 10X DNase I Reaction Buffer (100 mM Tris-HCl, 25 mM $MgCl_2$, 5 mM $CaCl_2$, pH 7.6)
2. Add 2 units of DNase I, mix thoroughly and incubate at 37°C for 10 minutes.
3. Add 1 µl of 0.5 M EDTA (to a final concentration of 5 mM). Heat inactivate at 75°C for 10 minutes

Product diagram: (an innovative 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.

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