Total RNA, DNA and Protein Extraction Kit (Cat. RDP-01)

Description:

This kit is designed for rapidly isolating the highest yields and quality of total RNA, DNA and pure, native protein fragments from cells/tissue samples, offering a simple, fast, environmental-friendly protocol for total RNA/DNA/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, DNA footprinting, 1D/2D electrophoresis, western blotting, EMSA, Southern blotting, TF-TF interaction arrays and other protein/DNA assays. The kit is compatible with cells/tissues from LCM samples.

- **Key features:**

1. Obtain the highest yield and integrity of total RNA, DNA and pure, native proteins within 1.5 hour.
2. No filter column or vacuum filtration is required, able to avoid the loss of RNA/DNA during extracting.
3. Suitable for extracting the pure total RNA/DNA/protein from the small/tiny tissues or LCM samples.

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

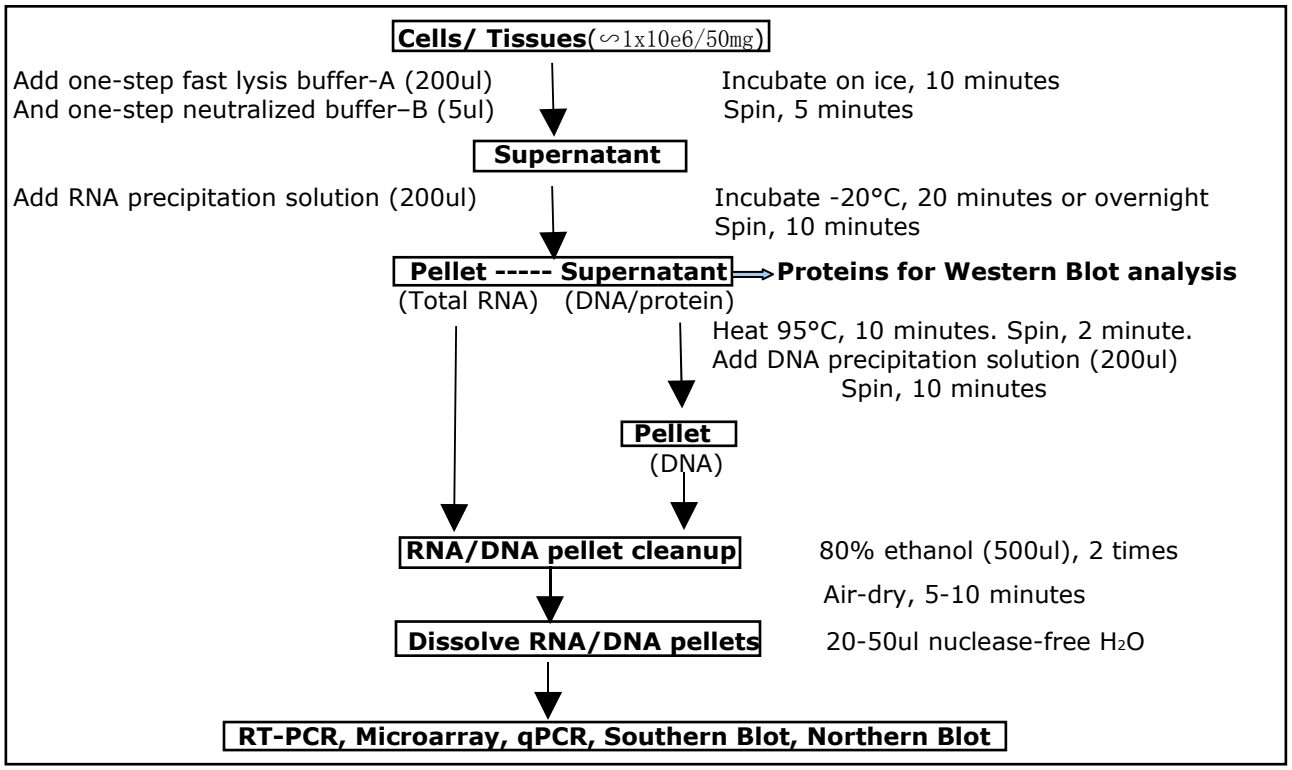
Components:	Quantity (50 extracts)	Storage
One-step Fast Lysis Buffer-A contains nuclease/protease inhibitors	10.0 mL	-20°C
One-step Neutralized Buffer-B	250 uL	-20°C
RNA Precipitation Solution (2x)	10.0 mL	-20°C
Protein Extraction Buffer -C contains protease/ phosphatase inhibitors	2.5 mL	-20°C
DNA Precipitation Solution (2x)	10.0 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/cells for 10-20 strokes may obtain higher yields of total RNA, DNA and proteins.
3. Centrifuge at 1,000 xg for 5 minutes at 4°C and transfer the supernatant into a clean 1.5mL tube. Discard the pellet (cells debris).
4. Add 5ul **one-step neutralized buffer-B** into the supernatant, mix well by mid-speed vortexing.
5. Centrifuge at 13,000 xg for 2 minutes at 4°C and transfer the supernatant into a clean 1.5mL tube. Save the pellet on ice and label as **Total DNA/protein**.
6. Add 200ul **RNA Precipitation Solution (2x)** into the supernatant, mix well and incubate at -20°C for 20 minutes or overnight.
7. Centrifuge at highest speed (∽ 14,000 xg) for 10 minutes at 4°C. The pellet is the total RNA and the supernatant contains DNA and protein fragments.
8. Transfer the supernatant into the pellet tube from **step 5**, and add 50ul **protein extraction buffer-C.** Incubate on ice for 10 minutes, vortex vigorously at highest speed for 20 seconds every 5 minutes.
9. Divide the suspension into two tubes: 200ul for DNA extraction, 200ul for protein assays. Keep tubes on ice. **This suspension contained total protein** is ready for most protein assays: 1D and 2D electrophoresis and Western blotting.
   Measure the protein concentration using a spectrometer and store the protein solution at -80°C.

10. Simply rinse the RNA pellet with 500ul ice cold 80% ethanol for 2 times without resuspending the RNA pellet. The RNA pellets will not be visible if the concentration is less than 20ng/ul. Air dry the pellets for 5-10 minutes or until no ethanol smells.
11. Dissolve the RNA pellets in 20 ul nuclease-free H_2O or TE if the RNA pellet is visible. Otherwise, use 10ul of nuclease-free H_2O or TE.
    Measure the RNA concentration with 260/280nm and store all the RNA extracts at -80°C.
12. DNA isolation and purification:

- Heat the suspension (200ul) prepared for DNA extraction from **step 9** at 95°C for 10 minutes.
- Centrifuge at 12,000 xg for 2 minutes at 4°C and transfer the supernatant into a clean tube.
- Add 200ul DNA precipitation solution (2x) into the supernatant, mix well by vortexing.
- Centrifuge at 12,000 xg for 10 minutes at 4°C. Aspirate liquid. **The pellet is the DNA.**
- Simply rinse DNA pellet with 500ul 80% ethanol for 2 times (don't resuspend the DNA pellets). Air-dry pellet for 5-10 minutes and dissolve DNA in 50ul TE buffer or distilled water.
  Measure the DNA Concentration with 260/280nm and store DNA at -20°C.
- Pipette 2-3ul of DNA solution into a 25ul PCR Master Mixture and run PCR/Real-Time PCR at thermal cyclers.

Flow Chart of RNA/DNA/protein Extraction: (an innovative RNA/DNA 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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