Total RNA and DNA Extraction Kit (Cat. VRD-01)

Description:

- This kit is designed for rapidly isolating the highest yields and quality of total RNA and DNA from cells/tissue samples, offering a simple, fast, environmental-friendly protocol for total RNA/DNA 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/DNA from small amounts of sample material for RT-PCR, qPCR, microarrays and other RNA/DNA assays. The kit is compatible with cells/tissues from LCM samples and urine.
- **Key features:**
  1. Obtain the highest yield and integrity of total RNA and DNA 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/DNA 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 inhibitors	2.5 mL	-20°C
One-step P-Lysis Buffer-B contains nuclease inhibitors	250 uL	-20°C
RNA Precipitation Solution (2x)	2.5 mL	-20°C
DNA Precipitation Solution (2x)	5.0 mL	-20°C

1. Prepare cells/samples from urine: Centrifuge urine samples (10-50ml) at 2000 xg for 5 minutes at 4°C. Carefully remove all the supernatant and add 1.0 mL 1xPBS to resuspend the pellet. Transfer all the suspension into a clean 1.5ml microcentrifuge tube and centrifuge at 2000 xg for 5 minutes at 4°C. Aspirate liquids and save pellet on ice for RNA/DNA extraction.

2. Prepare cells/samples from culturing cells: Harvest cells (∽1x10e6) as usual, and centrifuge at 1000 xg (or 3000rpm) for 5 minutes at 4°C. Carefully remove all the supernatant and add 1.0 mL 1xPBS to resuspend the pellet. Transfer all the suspension into a clean 1.5ml microcentrifuge tube and centrifuge at 1000 xg for 5 minutes at 4°C. Aspirate liquids and save pellet on ice for RNA/DNA extraction.

3. Add 50ul ice-cold **one-step fast lysis buffer-A** and 5ul **one-step P-Lysis buffer-B** to the pellet and incubate on ice for 10 minutes. Vortex vigorously at highest speed for 20 seconds every 5 minutes.
4. Centrifuge at highest speed (∽13,000 xg) for 5 minutes at 4°C and transfer the supernatant into a clean 1.5mL tube. Discard the pellet.
5. Add 50ul **RNA Precipitation Solution (2x)** into the supernatant, mix thoroughly by pipette up and down several times, and incubate at -20°C for 20 minutes or overnight.
6. Centrifuge at highest speed (∽13,000 x g) for 10 minutes at 4°C. The pellet is the total RNA and the supernatant contains DNA. Transfer the supernatant (DNA) into a new clean 1.5ml tube and keep the RNA pellet on ice or continue the step 8 for RNA cleanup.
7. DNA Extraction:

- Heat the supernatant (DNA) at 95°C for 5 minutes and centrifuge at highest speed (∽13,000 xg) for 1 minute at 4°C. Discard the pellet and transfer the supernatant into a new 1.5ml tube.

- Add 100ul **DNA Precipitation Solution (2x)** into the supernatant, mix thoroughly by pipette up and down several times and centrifuge at highest speed (∽13,000 xg) for 5 minute at 4°C. Carefully remove all the supernatant and save the pellet. The pellet is the DNA.

8. Simply rinse the RNA and DNA pellets with 500ul ice cold 70% ethanol for 2 times without resuspending the RNA/DNA pellet. Centrifuge at highest speed (∽13,000 xg) for 10 minute at 4°C if the pellets are resuspended. Air-dry the pellets for 5-10 minutes.

9. Dissolve the RNA and DNA 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/DNA concentration with 260/280nm.

10. Store all the RNA/DNA extracts at -80°C.

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