【Product Name】 HiPure Total RNA Kit

【Product specifications】 50 Preps/Kit

【Intended Use】

This product is suitable for rapid extraction of RNA (include miRNA) from tissue, cells, blood, s and other clinical samples. RNA can be used directly for RT-PCR, quantitative RT-PCR, test of virus DNA and so on.

Principle

This product is based on silica column purification. Remove paraffin by Buffer DPS. Sample lysis with proteinase K digestion requires only 15 minutes. After lysis, samples are incubated at 80°C for 15 minutes. Transfer to an adsorption column and RNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, RNA was finally eluted with low-salt buffer.

【Main Composition】

Cat.No.	IVD4121	Main Composition
HiPure DNA Mini Column II	50	Silica Column
HiPure RNA Mini Column	50	Silica Column
2ml Collection Tubes	150	PP Column
Proteinase K	24 mg	Proteinase K
Protease Dissolve Buffer	1.8 ml	Glycerol/Tris/CaCl2
DNase I	600 μl	DNase I
DNase Buffer	6 ml	Tris/CaCl2
RTL Lysis Buffer	40 ml	Guanidine Salt
RNA Digestion Buffer	15 ml	Guanidine Salt
Buffer RWC*	20 ml	Guanidine Salt
Buffer RW2*	20 ml	Tris/NaCl
Nuclease Free Water	10 ml	DEPC-Treated Water

【Storage conditions and Validity】

Proteinase K should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, Proteinase K up to 8 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

【Preparation before Use】

- Dilute Buffer RWC with 40 ml absolute ethanol and store at room temperature.
- Dilute Buffer RW2 with 80 ml absolute ethanol and store at room temperature
- Add 1.2ml Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- (Optional) Add 20μl 2-mercaptoethanol (or 2M DTT)per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature.

A: Sample Prepare

a. **Cells Grown in Suspension (no more than 5 x 10^6 cells)**

Pellet cells by centrifugation. loosen the cell pellet thoroughly by flicking the tube. Add 500μl of RTL Lysis Buffer and vortex vigorously. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.

b. **Cells Grown in Monolayer (no more than 5 x 10^6 cells)**

Lyse cells directly in a culture dish by adding 600μl of RTL Lysis Buffer and passing the cell lysate several times through a blue pipette tip. Always use more RTL Lysis Buffer if in the lysate is too viscous to aspirate with a pipette. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.

c. **Whole Blood (Do not use more than 1.5ml blood)**

Separate the leukocytes cell from 0.5~1.5ml Whole blood. Resuspend the leukocytes pellets completely by 50μl Buffe PBS or water. Add 500μl of RTL Lysis Buffer and vortex vigorously to lyse. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.

d. **Animal Tissue (Do not use more than 20 mg Tissue)**

Homogenize no more than 20mg tissue samples in 600μl RTL Lysis Buffer using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and /pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube and add RTL Lysis Buffer. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe. After lysate, centrifuge at 14,000 x g for 3 minute at room temperature.

e. **Fiber-Rich Tissue (Do not use more than 30 mg Muscles, Skin, Esophagus, Heart)**

Homogenize no more than 30mg tissue samples in 600μl RTL Lysis Buffer using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube and add RTL Lysis Buffer. Transfer 500μl lysate to 1.5ml tube. Add 250μl RNA Digestion Buffer and 20μl Proteinase K to the samples. Mix well and Incubate at 55°C for 15min. Centrifuge at 14,000 x g for 3 min.
Insert a HiPure DNA Mini Column in a 2ml Collection Tube. Transfer the supernatant to a the DNA Mini column. Centrifuge for 60 s at ⩾12000 x g . Discard the column, and save the flow-through. Proceed to step 2(B1) or Proceed to step 3 (B2).

f. **Trizol/MagZol Regeant (without chloroform extraction):**
Following Trizol/MagZol Reagent protocol, add 600µl MagZol Reagent to lyse the sample. After lysate, centrifuge at 12,000 x g for 10 minutes at 2-8°C.

g. **Trizol/MagZol Regeant (witht chloroform extraction):**
Following Trizol/MagZol Reagent protocol, add 1ml Trizol/MagZol Reagent to lyse the sample. After lysate, add 200µl chloroform to the lysate and centrifuge at 12,000 x g for 15 minutes at 2-8°C. Proceed to step 2(B1) or Proceed to step 3 (B2).

B: Column Protocol

1. Insert a HiPure DNA Mini Column in a 2ml Collection Tube. **Transfer the homogenized lysate or supernatant to a the DNA Mini column.** Centrifuge for 60 s at ⩾12000 x g . Discard the column, and save the flow-through.
Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

B1: Large RNA(>200nt) Protocol:

2. **Add 1 volume 70% ethanol to the flow-through and mix well by pipetting or vortexing**.
If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly. When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure. For maximum RNA yields from liver, use 50% ethanol instead of 70% ethanol.
3. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.
4. **Add up to 700µl of the sample from Step 2 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
5. Repeat Step 4 until all of the sample has been transferred to the column. Proceed step 6.

B2: Total RNA(Include miRNA) Protocol:

2. **Add 250µl RNA Digestion Buffer and 20µl Proteinase K to the flow-through.** Mix well and incubate at 55°C for 15min. Centrifuge at 14,000 x g for 3 min.
3. Transfer 600µl supernatant to a new 2ml microcentrifuge tube. Add 1.5 volume absolute ethanol to the supernatant by pipetting or vortexing.
4. Insert a HiPure RNA Mini Column in a 2ml Collection Tube. **Add up to 700µl of the sample from Step 3 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
5. Repeat Step 4 until all of the sample has been transferred to the column.
6. **Add 500µl Buffer RWC to the column, centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
7. **Add 10 µl DNase I to 90 µl DNase Buffer.** Mix by gently inverting the tube and centrifuge briefly to collect residual liquid from the sides of the tube.
8. **Add the DNase I incubation mix (100 µl) directly to the RNA Mini column membrane and place on the benchtop (15–25°C) for 15 min.**
Note: Be sure to add the DNase I incubation mix directly to the column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.
9. **Add 500 µl Buffer RWC to the column. Incubate at room temperature for 2min.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
10. **Add 500µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
11. **Add 500µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
12. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
13. Transfer the Column to a clean 1.5ml microcentrifuge tube. **Add 30~100µl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature. Store RNA at -20°C.
If the expected RNA yield is >30 µg, repeat step 13 using another 30–100µl RNase-free water, or using the eluate from step 13.