

【Product Name】 HiPure Total RNA Kit

【Product specifications】 50 Preps/Kit

【Intended Use】

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

Principle

This product is based on silica column purification. The sample is lysed and digested by Buffer RTL and Proteinase K. DNA/RNA is released into the lysate. DNA is removed by DNA column absorption. After adding ethanol, RNA is adsorbed onto the RNA column membrane, while protein is not adsorbed and is removed with filtration. Proteins and other impurities are washed by washing buffer RWC, and salts are washed by washing buffer RW2. RNA is finally eluted by elution buffer NFW.

【Main Composition】

Cat.No.	IVD4121-20	IVD4121	Main Composition
HiPure DNA Mini Column II	20	50	Silica Column
HiPure RNA Mini Column	20	50	Silica Column
2ml Collection Tubes	60	150	PP Column
Proteinase K	12 mg	24 mg	Proteinase K
Protease Dissolve Buffer	1.8 ml	1.8 ml	Glycerol/Tris/CaCl ₂
DNase I	600 µl	600 µl	DNase I
DNase Buffer	6 ml	6 ml	Tris/CaCl ₂
RTL Lysis Buffer	20 ml	40 ml	Guanidine Salt
RNA Digestion Buffer	5 ml	15 ml	Guanidine Salt
Buffer RWC*	10 ml	20 ml	Guanidine Salt
Buffer RW2*	10 ml	20 ml	Tris/NaCl
Nuclease Free Water	10 ml	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 24 months under these conditions.

【Preparation before Use】

- Dilute Buffer RWC with 20ml (20 Preps) or 40ml (50 Preps) absolute ethanol and store at room temperature.
- Dilute Buffer RW2 with 40ml (20 Preps) or 80ml (50 Preps) absolute ethanol and store at room temperature
- Add 0.6ml (20 Preps) or 1.2ml (50 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8°C 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 (≤ 5 × 10⁶ 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 Syringe.

b. Cells Grown in Monolayer (≤ 5 × 10⁶ 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 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 Syringe.

c. Whole Blood (≤ 1.5ml blood)

Separate the leukocytes cell from 0.5~1.5ml Whole blood. Resuspend the leukocytes pellets completely by 50µl Buffer 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 Syringe.

d. Animal Tissue (≤ 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 Syringe. After lysate, centrifuge at 14,000 × g for 3 minute at room temperature.

e. Fiber-Rich Tissue (≤ 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 × g for 3 min. Insert a HiPure DNA Mini Column in a 2ml Collection Tube. Transfer the supernatant to the DNA Mini column. Centrifuge for 60 s at ≥ 12000 × g. Discard the column, and save the flow-through.

Proceed to step 2(B1) or Proceed to step 3 (B2).

f. Trizol/MagZol Reagent (without chloroform extraction):

Following Trizol/MagZol Reagent protocol, add 600µl MagZol Reagent to lyse the sample. After lysate, centrifuge at 12,000 × g for 10 minutes at 2-8°C.

g. Trizol/MagZol Reagent (with 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 × 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 the DNA Mini column.** Centrifuge for 60 s at $\geq 12000 \times g$. 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 × 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.