FastPure Cell/Tissue Total RNA Isolation Kit V3

RC122

Vazyme

Instruction for Use

Version 26.1

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

This kit enables a rapid extraction of total RNA from animal tissues and cells. It is based on silica membrane purification technology and allows the extraction of high-quality RNA without the use of toxic reagents such as β-mercaptoethanol or phenol/chloroform. The lysis capability of Buffer RL in combination with Proteinase K ensures compatibility with a wider range of complex samples and supports higher input amounts. FastPure gDNA-Filter Columns III efficiently remove impurities and gDNA, while FastPure RNA Columns IV enable efficient RNA binding. Together with optimized buffers, high-purity total RNA can be obtained, with minimal residual gDNA and free of protein or other contaminants. It is suitable for a variety of downstream experiments including RT-PCR, RT-qPCR, and microarray analysis.

02/Components

	Components	RC122-01 (100 rxns)
	Buffer RL	45 ml
	Buffer BD	15 ml
	Buffer RW1	75 ml
	Buffer RW2	40 ml
	RNase-free ddH_2O	25 ml
■	Proteinase K	1 ml
	FastPure gDNA-Filter Columns III (each in a 2 ml Collection Tube)	2 × 50
	FastPure RNA Columns IV (each in a 2 ml Collection Tube)	2 × 50
	1.5 ml RNase-free Collection Tubes	2 × 50

Buffer RL & Proteinase K: Provide optimal conditions for efficient lysis of animal tissues and cells.

Buffer BD: Provides the optimal conditions required for RNA binding.

Buffer RW1: Removes impurities such as proteins and DNA.

Buffer RW2: Removes residual salts.

RNase-free ddH_2O: For elution of purified total RNA.

FastPure gDNA-Filter Columns III: Bind DNA and remove lysis impurities.

FastPure RNA Columns IV: Enable specific, high-affinity binding of total RNA.

2 ml Collection Tubes: Collect flow-through.

1.5 ml RNase-free Collection Tubes: Collect RNA.

03/Storage

Store at 15 ~ 25°C and ship at room temperature.

04/Applications

10 - 30 mg animal tissue;
<5 × 10^6 cultured cells.

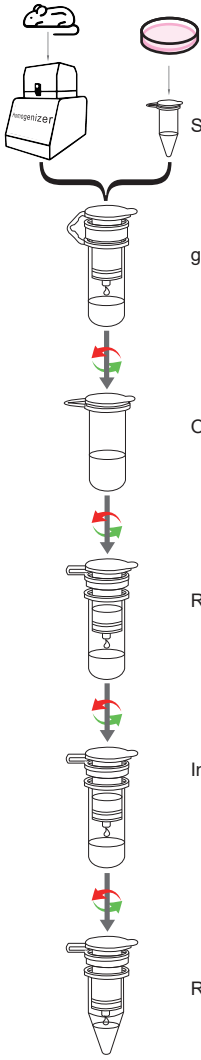
05/Self-prepared Materials

Anhydrous ethanol, 1.5 ml RNase-free centrifuge tubes, RNase-free pipette tips, RNase-Free DNase Set (Vazyme #RH104-C1) (Optional), etc.

06/Notes

1. Before first use of the kit, add the appropriate volume of anhydrous ethanol to Buffer BD and Buffer RW2 according to the label on the bottle, and mark the bottles.
2. Before use, check the Buffer RL for precipitate formation. If crystals are present, incubate at 37 °C until completely dissolved, then invert to mix thoroughly before use.
3. For optimal performance, limit sample input to 10 - 30 mg of tissue or <5 × 10^6 cells. For tissues with high nucleic acid content, such as spleen, thymus, do not exceed 10 mg. Otherwise, it may result in residual gDNA or low yield.
4. Use fresh samples. If extraction can not be performed immediately, rapidly freeze the samples in liquid nitrogen and store at −85 ~ −65 °C, avoiding repeated freeze-thaw cycles. To prevent RNA degradation, sample collection and storage should be performed as quickly as possible.
5. Incomplete disruption of samples may compromise RNA yield and easily cause column blockage. During homogenization, the temperature should be controlled to prevent RNA degradation caused by elevated temperature.
6. When using this kit, please wear a laboratory coat, disposable latex gloves and a disposable mask, and use RNase-free consumables to prevent RNase contamination.
7. All procedures must be performed at room temperature (15 ~ 25°C).

07/Mechanism & Workflow



Sample lysis: 350 µl Buffer RL + 10 µl Proteinase K, **incubate at room temperature for 5 min**

gDNA removal: Transfer the lysate to FastPure gDNA-Filter Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Collect the flow-through**

Optimize the binding conditions: Add 1 volume of Buffer BD to the flow-through (ensure that anhydrous ethanol has been added) or 1 volume of anhydrous ethanol, and mix thoroughly

RNA Binding: Transfer the entire mixture into FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the flow-through**

Impurities removal: Add 700 µl Buffer RW1, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the flow-through. Add 700 µl of Buffer RW2 (ensure that anhydrous ethanol has been added), centrifuge at 12,000 rpm (13,400 × g) for 30 sec, discard the flow-through, then centrifuge again at 12,000 rpm (13,400 × g) for 1 min and discard the collection tube

RNA Elution: Add 20 - 200 µl RNase-free ddH_2O, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 1 min

Fig 1. Workflow of the FastPure Cell/Tissue Total RNA Isolation Kit V3

08/Experiment Process

Please read this instruction carefully before starting the experiment.

1. Before first use of this kit, add appropriate volume of anhydrous ethanol to Buffer BD and Buffer RW2 according to the bottle labels, mark the bottles.
2. Before use, check the Buffer RL for precipitate formation. If crystals are present, incubate at 37 °C until completely dissolved, then invert to mix thoroughly before use.

08-1/Sample Pre-treatment

Animal tissue

◇ Homogenization: Add 350 µl Buffer RL per 10 - 30 mg of sample. Homogenize the sample using a glass or electric homogenizer until no visible tissue clumps remain. Add 10 µl Proteinase K, mix thoroughly, and **incubate at room temperature for 5 min**.

▲ Perform homogenization on ice to prevent RNA degradation caused by localized heating.

◇ Liquid nitrogen grinding: After grinding the sample with liquid nitrogen, immediately transfer to Buffer RL, adding 350 µl Buffer RL per 10 - 30 mg of pulverized sample. Vortex until no visible cell clumps remain. Then add 10 µl Proteinase K, mix thoroughly, and **incubate at room temperature for 5 min**.

Cell culture

◇ Adherent cells: No prior digestion required. Aspirate the culture supernatant, add Buffer RL directly to the culture vessel to detach and lyse the cells immediately. Alternatively, collect trypsin-digested cells by centrifugation and add 350 µl Buffer RL per $<5 \times 10^6$ cells. Vortex until no visible cell clumps remain. Then add 10 µl Proteinase K, mix thoroughly and **incubate at room temperature for 5 min**.

◇ Suspension cells: Collect the cells directly by centrifugation. Add 350 µl Buffer RL per $<5 \times 10^6$ cells and vortex until no visible cell clumps remain. Then add 10 µl Proteinase K, mix thoroughly, and **incubate at room temperature for 5 min**.

▲ When processing multiple tissue or cell samples, several volumes of Buffer RL with Proteinase K can be pre-mixed and used immediately; This will not significantly affect Proteinase K activity for a short period.

▲ If excessive foaming occurs in lysed tissue or cell samples due to vigorous shaking, do not discard the foam. Centrifuge at 12,000 rpm (13,400 × g) for 1 - 2 min to eliminate the foam before transfer.

08-2/RNA Extraction

Perform the following steps in an RNase-free environment.

1. Transfer the lysate to FastPure gDNA-Filter Columns III (put in a collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the FastPure gDNA-Filter Columns III and collect the flow-through.
2. Based on the input amount and characteristics, add 1 volume of Buffer BD (ensure that anhydrous ethanol has been added) or 1 volume of anhydrous ethanol to the flow-through, and mix thoroughly.

▲ After adding Buffer BD, the solution may become turbid or form flocculent precipitate, as expected during normal use. Mix the solution well by vortexing and proceed directly to the next step.

Sample Type	Examples	Input Volume	Recommended Binding Buffer	
			Buffer BD	Anhydrous ethanol
Cell	Mouse primary splenocytes, HEK293 cells, HeLa cells, etc.	<1 × 10^6 cells		√
		1 × 10^6 - 5 × 10^6 cells	√	
Low nucleic acid content tissues	Heart, muscle, skin, adipose tissue, brain, bone, etc.	<10 mg		√
		10 - 30 mg	√	
High nucleic acid content tissues	Liver, spleen, lung, kidney, thymus, intestine, etc.	<10 mg	√	
		10 - 30 mg	√	

3. Transfer the entire mixture from Step 2 to FastPure RNA Columns IV (put in a collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the flow-through.

4. DNase I Digestion (Optional): FastPure gDNA-Filter Columns III can efficiently remove most gDNA. For further gDNA clearance, perform digestion with RNase-Free DNase Set (Vazyme #RH104-C1) as follows:
   a. Add 400 µl Buffer RW2 (ensure that anhydrous ethanol has been added) to the FastPure RNA Columns IV, centrifuge at 12,000 rpm (13,400 × g) for 30 sec and discard the flow-through.
   b. Add 80 µl DNase I working solution dropwise to the center of the FastPure RNA Columns IV without touching the membrane, and incubate at room temperature for 15 min.

5. Add 700 µl Buffer RW1 to the FastPure RNA Columns IV, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the flow-through.

6. Add 700 µl Buffer RW2 (ensure that anhydrous ethanol has been added) to the FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the flow-through.

7. Place the FastPure RNA Columns IV back into the collection tube. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to remove residual ethanol and prevent carryover.

8. Transfer the columns to a new 1.5 ml RNase-free Collection Tubes carefully. Add 20 - 200 µl of RNase-free ddH_2O dropwise to the center of the columns without touching the membrane. Incubate at room temperature for 1 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to elute the RNA.
   ▲ To maximize RNA yield, pre-warm RNase-free ddH_2O to 65°C. After applying to the membrane, incubate at room temperature for 2 - 5 min. Alternatively, a second elution can be performed following the initial centrifugation step.

9. The purified total RNA can be used directly for downstream experiments or stored at -85 ~ -65°C.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Column clogging	1. Excessive sample input	For routine samples, do not exceed 30 mg input. For tissues with high DNA/RNA content (e.g., spleen, thymus), do not exceed 10 mg.
	2. Insufficient grinding or homogenization	Increase grinding intensity, or centrifuge the lysate at 12,000 rpm (13,400 × g) for 5 min and proceed with the supernatant only.
No RNA obtained or low yield	1. Insufficient input amount	Increase sample input. Do not exceed 30 mg of tissue or 5 × 10^6 cells per preparation.
	2. Improper sample storage	RNA degradation due to endogenous RNase activity. Use fresh samples or samples stored at -85 ~ -65°C with no prior freeze-thaw cycles.
	3. Incomplete grinding or homogenization	Increase grinding intensity, or increase the volume of lysis buffer and extend the lysis incubation time.
	4. Improper elution	Add RNase-free ddH_2O onto the center of the membrane. Reduce elution volume as appropriate. Pre-warm the RNase-free ddH_2O to 65°C, and extend the incubation time or perform a second elution.
RNA degraded	1. The sample was not stored promptly	Use fresh sample or sample stored at -85 ~ -65°C that have never been thawed.
	2. Repeated sample freeze-thaw cycles	Avoid repeated freeze-thaw cycles. Aliquot samples into single-use volumes prior to storage.
	3. RNase contamination from electrophoresis or the work environment	Before the electrophoresis, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min and rinse it with RNase-free ddH_2O. Use RNase-free ddH_2O to prepare electrophoresis buffer to ensure the RNase-free environment.
Inhibition of downstream reaction or low purity	1. Residual salt ion carryover	Perform an additional wash step with Buffer RW2. To ensure complete salt removal, add Buffer RW2 to the walls of the spin column, close the cap, and invert the column 2 - 3 times before centrifugation to wash residual salts from the column walls.
	2. Residual ethanol carryover	After Step 7, leave the spin column cap open and air-dry for 2 - 5 min to allow complete evaporation of residual ethanol.
gDNA contamination	1. Excessive sample input	DNA/RNA content varies greatly among different samples. Do not exceed 30 mg of tissue or 5 × 10^6 cells. For tissues with high DNA content such as spleen and thymus, the input amount should not exceed 10 mg. For further gDNA removal, DNase I digestion can be performed according to Step 4 in Section 08-2/RNA Extraction.

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