FreeZol Reagent

R711

Instruction for Use

Version 23.2

Vazyme

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01/Product Description

FreeZol Reagent is widely applicable to the extraction of total RNA from cultured cells, animal tissues, and simple plant tissues. Compared with the conventional Trizol method, this product features a simple procedure that can be performed at room temperature with no need to use chloroform for phase separation. In addition, this product ensures the integrity and purity of the extracted RNA by precipitating proteins, DNA, polysaccharides and other impurities in the organic phase while retaining RNA in the upper aqueous phase. The whole procedure can be completed in 1 h. The obtained total RNA can be used directly for RT-PCR, qRT-PCR, Northern Blot, Dot Blot, in vitro translation, NGS, and other molecular biology experiments.

02/Components

Components	R711-01 (200 rxns)	R711-02 (400 rxns)
FreeZol Reagent	100 ml	200 ml
Dilution Buffer	40 ml	80 ml

03/Storage

Store at 2 ~ 8°C and transport at room temperature.

04/Applications

Animal/plant tissues (20 - 50 mg)

Cells (1×10^6 - 1×10^7)

05/Self-prepared Materials

Isopropanol, 75% ethanol (prepared with RNase-free ddH_2O), RNase-free ddH_2O.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. This product contains phenol, which is toxic and corrosive. Wear protective equipment, such as protective clothing, gloves, goggles, and face shields while handling the reagents. In case of eye contact, flush immediately with large amounts of water and seek medical attention. In case of skin contact, flush immediately with large amounts of detergent and water. If discomfort persists, seek medical attention.
2. The key to RNA extraction is preventing RNase contamination. RNase, which is prevalent in the environment and extremely stable, can rapidly degrade RNA even at trace amounts. Therefore, take all necessary precautions as per the conventional RNA extraction procedure, including wearing a mask and sterile disposable gloves, working in a separate clean area, and using RNase-free labware.

07/Mechanism & Workflow

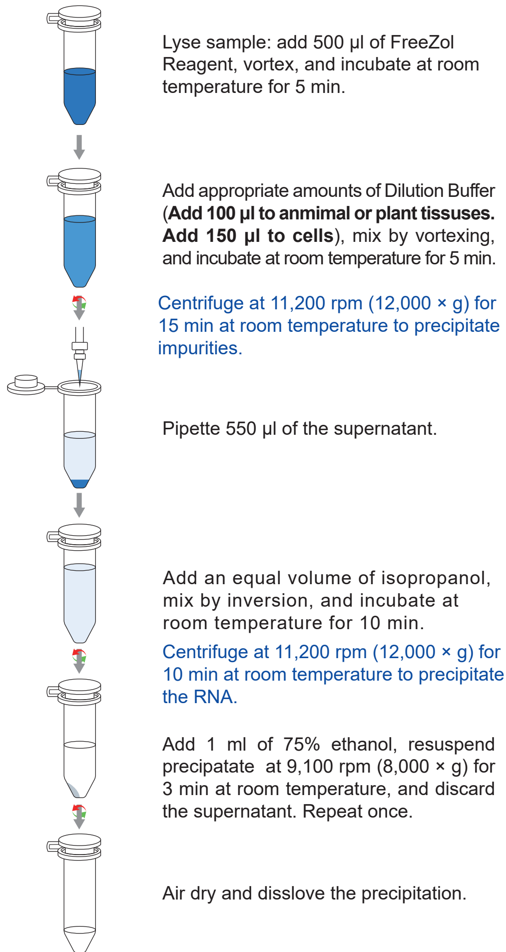

Fig 1. Workflow of FreeZol Reagent

08/Experiment Process

08-1/Sample Processing

◇ Animal/plant tissues

1. Flash freeze fresh tissue in liquid nitrogen, and then quickly transfer to a mortar precooled with liquid nitrogen. Grind with a pestle while adding liquid nitrogen until the sample is ground into powder (with no obvious granules).

▲ Insufficient grinding may impair RNA yield and quality.

2. Transfer the powdered sample into a centrifuge tube, add 500 μl of FreeZol Reagent per 50 mg of tissue, and vortex until the sample is completely lysed. Incubate at room temperature for 5 min.

▲ Up to 50 mg of animal/plant tissue can be lysed per 500 μl of FreeZol Reagent. Too much samples may lead to insufficient lysis and decrease product purity. Liver, spleen, kidney and other tissues are rich in DNA/RNA, and excessive sample inputs will lead to residual gDNA or low yield of RNA.

▲ If cryogenic grinding in liquid nitrogen is not feasible, mince the fresh tissue as finely as possible, immerse in the FreeZol Reagent, and homogenize in a high-speed electric homogenizer until the tissue pieces are completely lysed. Alternatively, fully immerse the fresh tissue in RNA Keeper Tissue Stabilizer (Vazyme #R501) to effectively inactivate the RNase before treating the sample with FreeZol Reagent and homogenizing it in an electric homogenizer until completely lysed.

3. (Optional) Centrifuge at 11,200 rpm (12,000 × g) for 5 min at room temperature. Carefully transfer the supernatant to a new 1.5 ml centrifuge tube. Do not disturb the precipitate.

▲ If the sample contains large amounts of protein, fat, polysaccharides, myofibers, or plant tubers, the insoluble substances can be removed by centrifugation. The precipitate contains cell membranes, polysaccharides, and high molecular weight DNA, while the RNA remains in the supernatant. When extracting RNA from tissue samples with high fat content, the top layer should be removed if it contains a large amount of oil. Proceed to the next step after transferring the supernatant.

◇ Suspension cells

1. Collect the cells by centrifugation, discard the supernatant, and add 500 μl of FreeZol Reagent per 1×10^6 - 1×10^7 cells.

2. Vortex or pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

▲ Frozen cells should be vortexed immediately after adding FreeZol Reagent to avoid incomplete lysis.

◇ Adherent cells

1. Discard the culture medium and rinse once with 1 × PBS.

2. Add 500 μl FreeZol Reagent to each well of a 6-well cell culture plate or a 3.5 cm diameter dish (approximately 10 cm^2 culture area), allow the FreeZol Reagent to fully cover the cell layer, and then detach the cells by pipetting.

▲ Firmly adherent cells (cell clumps) can be detached with a cell scraper or a clean pipette tip, or add the volume of FreeZol Reagent to1 ml. Alternatively, detach the cells using trypsin before adding FreeZol Reagent, and then follow the steps for suspension cells.

3. Transfer the mixture to a 1.5 ml centrifuge tube, vortex or pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

08-2/RNA Extraction

1. Add Dilution Buffer to the above lysates. **Animal or plant tissues**: Add 100 μl Dilution Buffer per 500 μl of FreeZol Reagent. **Cells**: Add 150 μl Dilution Buffer per 500 μl of FreeZol Reagent. Cap the centrifuge tube tightly and vortex thoroughly until the solution becomes a homogeneous emulsion. Incubate at room temperature for 5 min.

▲ Ensure that the solution is thoroughly mixed to yield a homogeneous emulsion, as insufficient mixing may impair the efficiency of RNA extraction and impurity removal.

2. Centrifuge at 11,200 rpm (12,000 × g) for 15 min at room temperature.
3. Carefully take out the centrifuge tube. Carefully transfer the supernatant (about 500 μl) into a new centrifuge tube.
   ▲ The upper aqueous phase, which takes up about 90% of the total volume, is approximately 550 μl if 500 μl of FreeZol Reagent is used for the extraction. Absorbing the underlying pellet will lead to genomic and impurity contamination.
   ▲ The supernatant may be slightly turbid or colored for some samples, which will not affect the product yield or purity. It is safe to proceed to the following steps.
   ▲ When the amount of tissue input is 50 mg, the volume of the supernatant solution is recommended to be reduced to 450 μl and avoid transferring any of the underlying pellet into the pipette.
4. Add an equal volume of isopropanol to the obtained supernatant. Mix well by inversion and incubate at room temperature for 10 min.
5. Centrifuge at 11,200 rpm (12,000 × g) for 10 min at room temperature. A white, gel-like pellet can be seen at the side and bottom of the tube. Carefully discard the supernatant, taking care not to lose the pellet.
   ▲ Low content of RNA will lead to inconspicuous pellet, so be careful to discard the supernatant to avoid losing the pellet.
   ▲ Some special samples, such as rice leaf. The RNA pellet will disperse on the wall of the centrifuge tube, resulting in inconspicuous white pellet. Discard the supernatant carefully, and the pellet will be visible after centrifugation in step 7.
   ▲ To reduce the residue of impurities, discard the supernatant as far as possible in this step and do not lose the pellet.
6. Add 1 ml of 75% ethanol (prepared with RNase-free ddH_2O). Gently flick the tube to resuspend the pellet, and invert the tube a few times.
7. Centrifuge at 9,100 rpm (8,000 × g) for 3 min at room temperature. Discard the supernatant, taking care not to lose the pellet.
8. Repeat Step 6 and 7 once. Discard the supernatant.
   ▲ To minimize residual impurities, discard as much supernatant as possible. It is recommended to discard most of the supernatant, spin the tube briefly to collect all the liquid, and then remove the remaining liquid with a pipette, taking care not to disturb the pellet.
9. Air dry at room temperature. Add 20 - 100 μl of RNase-free ddH_2O to dissolve the pellet and vortex at room temperature for 3 min (or pipette up and down) for thorough dissolve. The RNA can be aliquoted and stored at -85 ~ -65°C for long-term storage or -30 ~ -15°C for short-term storage.
   ▲ Air dry the RNA pellet for 2 - 3 min. Do not over dry the pellet because completely dry RNA will be difficult to resuspend.
   ▲ The RNA should be completely resuspended, or the concentration quantitation may be inaccurate.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Incomplete dissolution of RNA	1. Prolonged drying	Avoid excessive drying after washing with 75% ethanol.
	2. Too much product	Increase the volume of RNase-free ddH_2O, increase dissolve time, or incubate in a 55 ~ 60°C water bath for 2 - 3 min.
	3. Too many impurities	After the sample is completely lysed, centrifuge the sample and use the supernatant for subsequent steps.
RNA degradation	1. RNase contamination	Ensure all centrifuge tubes, pipette tips, and relevant solutions are free from RNase contamination. Take appropriate preventive measures, including wearing masks and sterile disposable gloves and working in a separate clean area.
	2. Improper or prolonged sample storage	Use a fresh sample or a sample flash frozen in liquid nitrogen and stored at -85 ~ -65°C.
	3. Repeated freezing and thawing of the sample	Store samples in aliquots to avoid degradation caused by repeated freezing and thawing. Add lysis buffer immediately to samples retrieved from liquid nitrogen and mix thoroughly to avoid RNA degradation caused by prolonged exposure to room temperature or incomplete mixing with the FreeZol Reagent.
	4. Electrophoresis issues	Before running the gel, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min or treat with RNase, RNA, and DNA remover (Vazyme #R504) for 5 min, and then rinse with RNase-free ddH_2O. Prepare the electrophoresis buffer with RNase-free ddH_2O. Use a new Loading Buffer.
Inhibition downstream or low purity	1. Protein contamination	Reduce the sample input amount. Increase the volume of FreeZol Reagent.
	2. Polysaccharide contamination	Reduce the sample input amount.
	3. Fat contamination	After 08-1/Sample Processing/(Optional) Step 3, transfer the clear interphase layer for the next step.
	4. Residual salt	Increase the number of washes with 75% ethanol.
Genomic DNA Contamination	1.Excessive sample input amount.	Reduce the sample input amount.
		Increase the volume of FreeZol Reagent.
		For sample lysis, add an appropriate amount of HAc (5 μl per 500 μl of FreeZol Reagent).
		For reverse transcription, select a reverse transcription reagent containing a genome removal module, HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme #R323) is recommended.
		Design transintronic primers in order to avoid the involvement of the genomic DNA template in the amplification reaction.
Pellet is invisible after adding isopropanol and centrifug-ing	1. Low sample input or RNA content	After adding isopropanol, incubate at 2 ~ 8°C or -30 ~ -15°C for 10 - 30 min before centrifugation.
	2. Pellet lost	Discard the supernatant by pipetting instead of decanting, taking care not to lose the pellet.
	3. Too many metabolites in the sample	The pellet is dispersed on the wall of the centrifuge tube, so the supernatant should be slowly withdrawn with the pipette along the surface of the liquid (See 08-2/RNA Extraction/Step 5).
Storage time at each stage	1. After mixing with FreeZol Reagent	Store at 4°C for 12 h or -20°C for one week.
	2. After adding 75% ethanol	Store at 4°C for 12 h or -20°C for 72 h.

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