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Product Name: TRIzol NC Reagent
Cat. No.: GK20010

Components

Components	100 rxns	200 rxns
TRIzol NC Reagent	50ml	100ml
Dilution Buffer	20ml	40ml

Features

Applications	• Safe and low in toxicity: No need for toxic and harmful reagents such as chloroform and β-mercaptoethanol • Ease to use: RNA extraction in a monophasic system, no phase separation, all operations at room temperature • High RNA quality: High purity and integrity of RNA at a yield comparable to the traditional Trizol method
Shipping	Ship with blue ice.
Storage	Store at 2-8°C, protected from light for 1 years.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

Self-provided reagents

Chloroform, isopropanol, 75% ethanol (prepared with RNase-free ddH $_2$O), RNase Free H_2O.

I Preparation before experiment

The key to RNA preparation is to inhibit RNA degrading enzymes in cells and prevent contamination of RNA degrading enzymes in the equipment and reagents used. Therefore, the following measures must be taken in the experiment: wear disposable clean gloves and mask; use a special experimental bench for RNA operation; avoid talking during the operation, etc. The above methods can prevent the contamination of the experimenter's sweat and saliva by RNA degrading enzymes.

Cautions:

1. Try to use disposable enzyme-free sterile plastic consumables. If glassware is used, it should be treated with 0.1% DEPC aqueous solution at 37°C for 12 hours before use, and then autoclaved at 120°C for 30 minutes to inactivate residual DEPC.

2. Reagents used for RNA experiments must be sterilized by dry heat (180°C, 60min) or in glass containers after DEPC water treatment and sterilization using the above methods (disposable plastic

containers for RNA experiments can also be used) , The sterile water used must be treated with 0.1% DEPC and then autoclaved.

3. Reagents and sterile water for RNA experiments should be dedicated to avoid cross-contamination after mixing.

4. This product is only for scientific research use, should not be used for clinical medical diagnosis and other irrational purposes.

5. This product contains phenol, which is toxic and corrosive. Protective items should be worn when using, such as protective clothing, gloves, eye masks, face masks, etc. In case of contact with eyes, rinse immediately with plenty of water and go to the hospital for treatment. In case of contact with skin, please rinse immediately with a large amount of detergent and water, if you still feel unwell, please go to the hospital for treatment.

II Experiment Process

1. Sample Processing

1.1 Animal/plant tissues

1.1.1 Flash freeze fresh tissue in liquid nitrogen, and then quickly transfer to a mortar precooled with liquid nitrogen. Grind quickly with a pestle while adding liquid nitrogen until the sample is ground into powder thoroughly.

Note: Insufficient grinding may impair RNA yield and quality.

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

Note: Up to 50mg of animal/plant tissue can be lysed per 500 μL of TRIzol NC 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.

Note: If cryogenic grinding in liquid nitrogen is not feasible, mince the fresh tissue as finely as

possible, immerse in the TRIzol NC Reagent*, and homogenize in a high-speed electric homogenizer until the tissue pieces are completely lysed.*

1.1.3 (optimize) Centrifuge at 11,200rpm (12,000xg) at room temperature for 5min. Carefully transfer supernatant into new 1.5ml centrifuge tube, do not absorb precipitate.

Note: If the sample contains large amounts of protein, fat, polysaccharide or muscle fiber, plant tuber, etc., the insoluble substances can be removed by centrifugation. The precipitation obtained by centrifugation contains cell membrane, polysaccharide, high molecular weight DNA, while the RNA remains in the supernatant. When extracting tissue samples with a high fat content, if the upper layer contains a large amount of oil, it should be removed. Proceed to the next step after transferring the supernatant.

1.2 Suspension cells

1.2.1 Collect the cells by centrifugation, discard the supernatant thoroughly, and add 500μL of TRIzol NC Reagent per 1×10^6 - 1×10^7 cells.

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

Note: For frozen cells, the addition of TRIzol NC Reagent should be immediately vortexed to avoid incomplete lysis.

1.3 Adherent cells

1.3.1 Discard the culture medium and wash once with 1×PBS.

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

Note: Firmly adherent cells (cell clumps) can be detached with a cell scraper or a clean pipette tip, or add the volume of TRIzol NC Reagent up to 1mL. Alternatively, detach the cells using trypsin before adding TRIzol NC Reagent, and then follow the steps for suspension cells.

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

2. RNA Extraction

2.1 Add Dilution Buffer to the above lysates.

2.1.1 Animal/plant tissues: Add 100μL Dilution Buffer per 500μL of TRIzol NC Reagent. Vortex thoroughly until the solution becomes a homogeneous emulsion. Incubate at room temperature for 5min.

2.1.2 Cells: Add 150μL Dilution Buffer per 500μL of TRIzol NC Reagent. Cap the centrifuge tube tightly and vortex thoroughly until the solution becomes a homogeneous emulsion. Incubate at room temperature for 5min.

Note: Ensure that the solution should be fully mixed into a uniform solution, otherwise will impair the efficiency of RNA extraction and impurity removal.

2.2 Centrifuge at 11,200rpm (12,000×g) for 15min at room temperature.

2.3 Take out the centrifuge tube Carefully. At this time, impurities such as protein, DNA and polysaccharide in the sample precipitate to the bottom of the tube, RNA is distributed in the supernatant, and transfer the supernatant solution (about 500μL) carefully into a new centrifuge tube.

Note: The upper aqueous phase, which takes up about 90% of the total volume, is approximately 500μL if 500μL of TRIzol NC Reagent is used for the extraction. Absorbing the underlying pellet will lead to genomic and impurity contamination.

Note: 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.

Note: When the amount of tissue input is 50mg, the volume of the supernatant solution is recommended to be reduced to 450μl and avoid transferring any of the pellet.

2.4 Add an equal volume of isopropanol to the obtained supernatant. Mix well by inversion and incubate at room temperature for 10min.

2.5 Centrifuge at 11,200rpm (12,000×g) for 10min at room temperature. Then, gel-like RNA pellet can be seen at the side and bottom of the tube. Carefully discard the supernatant, be care not to lose the pellet.

Note: Low content of RNA will lead to pellet invisible, so be careful to discard the supernatant to avoid losing the pellet.

Note: To reduce the residue of impurities, discard the supernatant as far as possible in this step and do not lose the pellet.

2.6 Add 1mL of 75% ethanol (prepared with RNase-free ddH $_2$O). Gently flick the tube to resuspend the pellet, and invert the tube a few times.

2.7 Centrifuge at 11,200rpm (12,000xg) at room temperature for 3min. Discard the supernatant, be care not to lose the pellet.

2.8 Repeat Step 6 and 7 once. Discard the supernatant.

Note: In order to reduce the residual supernatant, the supernatant should be discarded as much as possible. It is recommended to discard most of the supernatant, briefly centrifuge all the liquid to the bottom of the tube, and then use a 10μL pipette to suck off the remaining liquid, taking care not to loss pellet.

2.9 Air dry at room temperature in clean bench. Add 20-100μL of RNase-free ddH $_2$O to dissolve the pellet and vortex at room temperature for 3 min (or pipette up and down) for dissolve thoroughly. The RNA can be aliquoted and stored at -85 ~ -65°C for long-term storage or -30 ~ -15°C for short-term storage.

Note: 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.

Ⅲ FAQ & Troubleshooting

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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	Recommended Operation 1.1.3 Optimal 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 TRIzol NC Reagent.

Caution: Product has not been fully validated for medical applications. For research use only.
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	4. Electrophoresis issues	1. Before running the gel, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min, and then rinse with RNase-free ddH_2O. Prepare the electrophoresis buffer with RNase-free ddH_2O. 2. Use a new Loading Buffer.
Inhibition downstream or low purity	1. Protein contamination	Reduce the sample input amount. Increase the volume of TRIzol NC Reagent.
	2. Polysaccharide contamination	Reduce the sample input amount.
	3. Fat contamination	Recommended Operation 1.1.3 Optimal Steps
	4. Residual salt	Increase the number of washes with 75% ethanol.
	5. Low A260/230	Increase the number of washes with 75% ethanol.
Genomic DNA	1.Excessive sample	Reduce the sample input amount.
		Increase the volume of TRIzol NC Reagent..
		For sample lysis, add an appropriate amount of HAc (5μl per 500μl of TRIzol NC Reagent.).

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Contamination	input amount.	For reverse transcription, select a reverse transcription reagent containing a genome removal module, SuperFast RT Master Mix for qPCR (gDNA remover) (Cat.No. GK10031) 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 centrifuging	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.
Storage time at each stage	1. After mixing with TRIzol NC Reagent	Store at 4°C for 12h or -20°C for one week.
	2. After adding 75% ethanol	Store at 4°C for 12h or -20°C for 72h.

Background

TRIzol NC Reagent is a total RNA extraction reagent that without using chloroform, and is widely

suitable for cultured cells, animal tissues, and plant tissues.

The extraction process takes about 1 hour, and the total RNA obtained can be directly used in molecular biology experiments such as RT-PCR, qRT-PCR, Northern Blot, Dot Blot, in vitro translation, and high-throughput sequencing.

It is recommended to use the SuperFast RT Master Mix for qPCR (gDNA remover) (Cat.No. GK10031) reverse transcription kit and SYBR Green qPCR Master Mix (Cat.No. GK10002) real-time fluorescence quantitative PCR kit for subsequent experiment.

Sample size and RNA yield		
Sample type	**Sample size**	**RNA yield**
Leukocyte	$1x10^6$ cells	10~20μg
Plant tissue	50mg	10~25μg
Cells	$1x10^6$ cells	10-30μg
Tissues such as muscle/brain	50mg	10-25μg
Liver	50mg	100~300μg

This product is a blue solution. During the RNA extraction process, impurities such as proteins, polysaccharides, and DNA can be precipitated to the bottom of the tube with an obvious blue precipitate layer, which is convenient for absorbing RNA supernatant, effectively removing impurities,

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therefore ensuring the integrity and purity of RNA. Compared with traditional TRIzol methods, The same 50mg tissue sample needs to add 0.5ml TRIzol NC Reagent, while the traditional TRIzol Reagent needs to add 1ml, so as it saves reagent. TRIzol NC Reagent is more easy to operate, can be operated at room temperature throughout the process, and does not require chloroform for phase separation, with higher security.

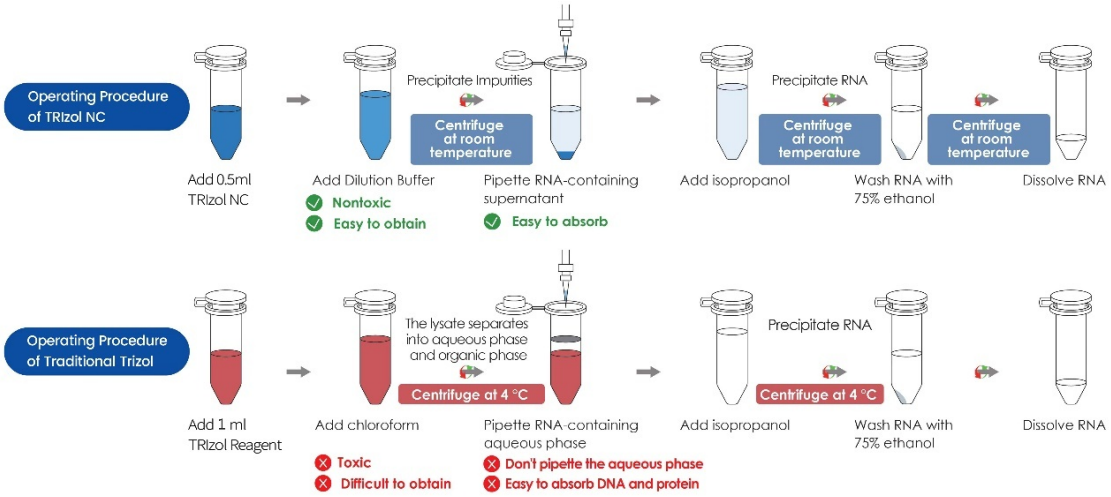

Fig1. Comparison of TRIzol NC operation process with conventional Trizol operation process

FAQ:

1.Q: The traditional TRIzol method requires centrifugation at 4°C for RNA extraction, but does this product extract RNA at room temperature?

A: Yes, TRIzol NC Reagent can extract RNA at room temperature, and the composition of the lysate can fully protect RNA from degradation. It is better if operated on ice and at 4 °C.

2.Q: Is there stratification after the TRIzol NC is added to the diluent and centrifuged?

A: After step 2.2 centrifugation, obvious blue bottom precipitate and RNA supernatant can be seen.

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3.Q: There was no pellet after adding isopropyl alcohol?

A: Usually, a white gelatinous pellet can be seen after isopropyl alcohol precipitation. If the pellet is not visible, it may be due to low RNA content or low tissue dosage. It is recommended to increase the amount of sample and TRIzol NC Reagent. When the supernatant is abandoned in steps 2.5-2.8, the method of absorbing rather than dumping is adopted to avoid the loss of pellet; The upper solution was taken and 5-10μg RNase-free glycogen was added as carrier for co-precipitation with RNA. In addition, some tissue materials contain more metabolites, resulting in precipitation could not accumulate and dispersed on the centrifugal tube wall, at this time, please slowly absorb supernatant followed by the liquid surface, after 75% ethanol washing RNA precipitation, white pellet will become obvious.

4.Q: Which samples are suitable for the product?

A: TRIzol NC Reagent can extract cell samples, animal and plant tissue samples. Not applicable to blood samples, not applicable to animal white fat tissue. TRIzol LS Reagent (Cat.No. GK20009) is recommended for blood samples. TRIzol Reagent (Cat.No. GK20008) is recommended for animal white fat tissue samples.