Product Data Sheet

Product Name: TRIzol Reagent

Cat. No.: GK20008

Features

Applications	• Permits the isolation of RNA, DNA, and protein from the same sample • Offers superior lysis capability, even with difficult sample types • Optimized formulations and protocols for tissues, cells, serum, virus, and bacteria
Storage Conditions	Store at 2-8°C protected from light for 2 years.

Protocol

Self-provided reagents

Chloroform, isopropanol, 70% ethanol (DEPC water configuration), Rnase Free H2O.

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; 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 plastic utensils. 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 remove 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.

II Experimental operation

The usage of TRIzol Reagent is as follows

Sample type	Sample size	Usage of TRIzol
Adherent cultured cells	10 cm^2	1 mL
Suspension culture cells	$1x10^6$-$10x10^6$	1 mL
Common tissue samples (muscle, etc.)	50-100 mg	1 mL
Special tissue samples (liver, spleen, etc.)	30-50 mg	1-2 mL
Plant tissue	30-50 mg	1 mL
Leukocyte	$1x10^6$	1 mL

Sample size and RNA yield

Sample type	Sample size	RNA yield
Leukocyte	$1x10^6$	10~20 μg
Plant tissue	25 mg	10~20 μg
Cells	$1x10^6$	8~15 μg
Tissues such as muscle/brain	50 mg	10~25 μg
Liver	50 mg	100~300 μg

TRIzol usage instructions

	Adherent cells	Suspension cells, yeast, bacteria	Animal and Plant tissue
1. Sample pretreatment	Pour out the culture solution from every 10 cm2 of the cultured cells and wash them with PBS once to remove as much excess solution as possible.	Pour the suspension cultured cells together with the culture solution into a centrifuge tube, centrifuge at 8,000 rpm for 2 min, discard the supernatant, and add 50μl of sterile water to resuspend the cells until there is no obvious precipitation.	Transfer the sample to a mortar pre-cooled with liquid nitrogen, grind the tissue with a pestle, and continuously add liquid nitrogen in the meantime until it is ground into a powder.
2. Add TRIzol	Add 1 ml of TRIzol to distribute the lysate evenly on the cell surface, and then use a pipette to blow the cells off. Transfer the cell lysate to a 1.5ml EP tube.	Add 1ml TRIzol.	Add the ground tissue to a 1.5 ml EP tube containing 1 ml TRIzol.
3. Lysed sample	After adding TRIzol, immediately turn it upside down with wrist force until the cells and tissue powder are evenly dispersed without lumps. Leave it at room temperature for 5 minutes to completely separate the nucleic acid-protein complexes.		
4. Add Chloroform	Add 200 μl chloroform, shake vigorously with the wrist for 15 seconds, and leave it at room temperature for 2 minutes.		
5. Centrifugal layering	Centrifuge at 13,000 rpm for 10 minutes, and transfer 600 μl of colorless supernatant to a new 1.5EP tube.		
6. Add isopropanol	Add 600 μl of isopropanol to the above 600 μl of supernatant, turn it upside down several times vigorously with the wrist, and place it at -20°C for 5 minutes.		
7. Centrifugation of total RNA	Centrifuge at 13,000 rpm for 10 min, carefully discard the supernatant, and save the bottom total RNA pellet.		
8. Rinse total RNA	Add 1ml of 70% ethanol to each tube of the pellet, turn it upside down several times, and centrifuge at 13,000 rpm for 5 min. Carefully discard the supernatant and save the bottom RNA pellet.		
9. Repeat the rinse one more time	Repeat step 8 and wash again.		
10. Volatile residual ethanol	Pour off the washing solution, centrifuge again for a short time for 10 seconds, absorb the remaining washing solution with a 10 μl tip, and place it at room temperature to evaporate the ethanol (~20min).		
11. Dissolve total RNA	Add 20-100μl TE Buffer or RNase Free H2O to each tube to dissolve total RNA.		

Common problems

1. Low extraction rate. Possible reasons: (a. Sample lysis or homogenization is not complete; b. RNA precipitation is not completely dissolved)
2.A260/A280<1.65. Possible reasons: (a. When measuring the absorbance, the RNA sample was not dissolved in water, but dissolved in TE; b. The amount of tissue added when the sample was homogenized was too much; c. After stratification, the supernatant was less than 500μl; d. The organic phase was mixed in the water phase)
3. Excessive DNA contamination. Possible reasons: (a. The amount of reagents added during sample homogenization is too small or the amount of tissue is too much; b. The sample contains organic solvents) Solution: using this reagent usually genomic DNA contamination content <0.1ng/μl, if it is necessary to remove DNA contamination completely, please use Rnase Free DNase I (HaiGene: A3001) to digest and remove genomic DNA contamination. If you use the Gold Medal cDNA First Strand Reverse Transcription Kit (HaiGene: D0401) (HaiGene: D0401), there is no need to digest to remove genomic DNA contamination in advance. The kit contains reagents to remove genomic DNA contamination.

Background

TRIzol Reagent is a product that can extract RNA from animal and plant samples. The sample can be fully lysed in TRIzol Reagent. During the homogenization or lysis of the sample, it can maintain the integrity of the RNA, while lysing the cells and dissolving the cell contents. TRIzol Reagent has a strong broad spectrum and can be applied to the extraction of total RNA from various samples. The extraction process is convenient and fast, and the entire operation can be completed within one hour. This reagent can be used for small samples (50-100 mg tissue, $1x10^6$ cells) and also suitable for large samples (≥1g tissue, $>10^7$ cells). It is applicable to humans, animals, plant tissues, and bacteria, and can process a large number of different samples at the same time, and the entire extraction process can be completed within one hour. The isolated total RNA protein and DNA are extremely low in contamination, and can be used for Northern Blot, reverse transcription, polyA screening, in vitro translation, RNase protection analysis, and gene cloning.