Product Data Sheet

Product Name: TRIzol LS Reagent
Cat. No.: GK20009

Features

Applications	• Formulated for use with liquid samples such as serum and virus preparations • Facilitates recovery of RNA, DNA, and protein from a single liquid sample • Offers excellent lysis capability, even with difficult biological fluids
Shipping	Ship with blue ice.
Storage	Store at 2-8°C protected from light for 2 years.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

Self-provided reagents

Chloroform, isopropanol, 70% ethanol (DEPC water configuration), 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; 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 DEPC water treatment related containers. 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

Sample size and RNA yield

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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Sample type	Sample size	RNA yield
Human whole blood	250 μl	3~10 μg
Leukocyte	$1x10^6$	10~20 μg
Cells	$1x10^6$	8~15 μg
Tissues such as muscle/brain	25 mg	10~25 μg
Liver	25 mg	50~100 μg

TRIzol LS usage instructions

	Liquid samples: Anticoagulated whole blood, Aerum, Virus liquid	**Suspension cells, Yeast, Bacteria**	**Animal and Plant tissue**	**Adherent cells**
1. Sample pretreatment	For solid samples such as feces, the samples can be resuspended in PBS, homogenized, centrifuged at 3000 rpm for 5 minutes, and the supernatant is taken as the virus liquid sample. Other samples can be used directly.	If the cell content in the sample is low, the cells need to be precipitated by centrifugation and then resuspended in 250 μl sterile water before proceeding to the following operations.	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.	Pour out the culture solution from the cultured cells every 10 cm2 and wash them with PBS once to remove as much excess solution as possible.

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2. Add TRIzol LS	Take 250 µl liquid sample and add it to a 1.5ml EP tube containing 750 µl TRIzol-LS.	Add 750µl TRIzol-LS to 250µl liquid sample.	Add the ground tissue to a 1.5ml EP tube containing 750 µl TRIzol-LS.	Add 750 µl TRIzol-LS to evenly distribute the lysate on the cell surface, then use a pipette to blow the cells off and transfer them to a 1.5ml EP tube.
3. Lysed sample	After adding TRIzol-LS, immediately turn the wrist upside down 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 water	No additional water is required for liquid samples.	Add 250 µl Rnase Free H_2O, mix well, and let stand for 2 minutes.		

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5. Add Chloroform	Add 200 µl chloroform, shake vigorously with the wrist for 15 seconds, and leave it at room temperature for 2 minutes.
6. Centrifugal layering	Centrifuge at 13,000 rpm for 10 minutes, and transfer 600 µl of colorless supernatant to a new 1.5 ml EP tube.
7. 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.
8. Centrifugation of total RNA	Centrifuge at 13,000 rpm for 10 minutes, carefully discard the supernatant, and save the bottom total RNA pellet.

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9. Rinse total RNA	Add 1 ml of 70% ethanol to each tube of the pellet, turn it upside down several times, centrifuge at 13,000 rpm for 5 minutes, carefully discard the supernatant, and save the bottom RNA pellet.
10. Repeat the rinse one more time	Repeat step 9 to wash again.
11. 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).
12. Dissolve total RNA	Add 20-100 µl TE Buffer or RNase Free H_2O to each tube to dissolve total RNA.

Common problems

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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 when the water phase was absorbed)
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 to digest and remove genomic DNA contamination. If you use the Gold Medal cDNA First Strand Reverse Transcription Kit there is no need to digest to remove genomic DNA contamination in advance The kit contains reagents to remove genomic DNA contamination.

Background

TRIzol LS Reagent is a reagent specially used to extract high-quality RNA from liquid samples (such as bacteria, viruses, yeast, cells, blood, tissue suspension). Compared with TRIzol Reagent, the only difference is its component concentration. TRIzol LS Reagent is a concentrated reagent for extracting RNA from liquid samples, so the amount required to extract the same amount of sample is relatively small. The sample can be fully lysed in TRIzolLS 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. The extraction process is convenient and fast. The entire operation can be performed within one hour. carry out. The total RNA protein and DNA obtained by using TRIzol LS Reagent is minimally contaminated and can be used for subsequent analysis such as Northern Blot, reverse transcription, polyA screening, RNase protection analysis, and gene cloning.

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