Total RNA Isolation Kit

Catalog No.: K056

Size:50T

Storage: The kit should be stored at RT for one year.

Kit components:

Item	50T
Lysis buffer	16 mL
Binding buffer I	16 mL
Binding buffer II	38 mL
Washing buffer I	32mL
Washing buffer II	63 mL
Elution buffer	5 mL
spin colunms	50 sets

Introduction

RNA Isolation Kit with SpinColumn is a safe, rapid, convenient, stable, efficient and high-quality extraction kit for RNA with a length of more than 200 nucleotides (NT) from animal tissues or cultured animal cells based on centrifugation Column method.The extracted RNA greater than 200 nucleotides (also often referred to as total RNA) can be used for a variety of conventional purposes.

The RNA extracted by this kit can be used for reverse transcription, RT-PCR, qPCR, Northern, Dot Blot, purified mRNA, in vitro translation, RNase protection assay, cDNA cloning and other downstream experiments.It can also be used for gene expression microarray analysis, high throughput sequencing and other situations that require high RNA quality.

The basic process of RNA extraction with this kit is described as follows. The samples were rapidly lysed in the lysate to release total RNA, which was then specifically bound to the purification column. Genomic DNA and other components such as proteins were effectively removed by high-speed centrifugation, and impurities such as proteins and salts that were not specifically bound were fully removed by three washes. Finally, high-purity RNA was eluted with the eluent.

This kit is suitable for the extraction of RNA from fresh or frozen animal tissues or cells. The recommended cell dosage is 0.5 to 1 million (upper limit is 10 million), and the tissue dosage is 15 to 20mg(upper limit is 30mg).The upper limit varies for different tissues or cells, for example, the upper limit for mouse liver tissue is 30mg, but the upper limit for muscle tissue is 20mg.

This kit can be used for RNA extraction from 50 samples.

Precautions for Use

For RNase-rich samples (such as animal tissues),It is recommended to add Dithiothreitol (DTT) to the lysate to a final concentration of 40mM (e.g., 20µl 2M DTT to 1ml lysate) or β-mercaptoethanol to a final concentration of 1% (e.g., 10µl β-mercaptoethanol to 1ml lysate) before use.Lysates containing DTT or β-mercaptoethanol can be stored at room temperature for 1 month.

All reagents and consumables supplied with this kit are Rnase-free and should be handled with care to avoid contamination.All self-prepared reagents and consumables should also be Rnase-free.If RNase contamination is possible, consider soaking in 0.01% DEPC water overnight, then autoclaving and drying.Avoid breathing or speaking on the sample or kit consumables used to prevent RNase contamination.It is recommended to wear a disposable mask.

RNA extraction from frozen cells or tissues is usually less effective than that from fresh cells or tissues.Because during freezing and thawing of the cell or tissue some of the RNase in the cell or tissue is released and cleaves the RNA in the sample.

All operations with this kit are performed at room temperature without ice bath.All centrifugations were also performed at room temperature.

The waste liquid collection pipe should be used many times in a single extraction. Do not discard it halfway.

The binding solution and washing solution contain ethanol. After use, the cap must be tightened to prevent volatilization, and it must be stored and used in accordance with the relevant specifications for flammable reagents.

This product is only for scientific research by professionals, not for clinical diagnosis or treatment, not for food or medicine, and not for storage in ordinary houses.

For your safety and health, please wear a lab coat and disposable gloves.

Assay Procedure

1. Preparation of samples

Cell sample: Collect about 500,000 to 1 million cells.For the suspended cells, centrifuge 1000-2000×g for 1 min, discard the supernatant, add 300 µL lysate, gently blow 8-10 times until the solid suspension is dissolved and the solution is clarified.For adherent cells, after absorbing the culture medium, add 300 µL lysate, gently blow 5-10 times until the solid suspension is dissolved and the solution is clarified, then transfer to a clean centrifuge tube.

Tissue samples: 15-20mg animal tissue was ground to powder in liquid nitrogen and 600 µL lysate was immediately added.The tissue can also be placed in a 1.5-mL centrifuge tube, quickly added with 600µl of pre-cooled lysate in an ice bath, and homogenized with a miniature electric homogenizer, or with a regular glass homogenizer.After grinding or homogenizing, gently blow the homogenized solution 8-10 times and leave it at room temperature for 3-5 minutes.Then centrifuge at approximately 14,000×g for 2 min and transfer the supernatant to a new centrifuge tube.For tissues that are easy to be cleaved and homogenized sufficiently (such as liver tissue and brain tissue), Step 2 can be directly accessed without high-speed centrifugation.

2. Add equal volume of binding solution to lysate, gently invert and mix 3-5 times.At this time, sediment may be produced, which is a normal phenomenon.

3. Transfer the mixture (including sediment) to the purification column, centrifuge at 12,000×g for 30 seconds, and discard the liquid in the collection tube.

4. Add 600 μl of washing liquid I, centrifuge at 12,000 × g for 30 seconds, and discard the liquid in the collecting tube.

5. Add 600 μl washing solution II, centrifuge at 12,000 × g for 30 seconds, and discard the liquid in the collecting tube.

6. Repeat Step 5 once.

7. Centrifuge at the highest speed (about 14,000-16,000×g) for 2 minutes to remove the residual liquid.

8. Put the RNA purification column into the RNA elution tube provided by this kit, add 30-50 μL eluate, place it at room temperature for 2-3 minutes, centrifuge it at the highest speed for 30 seconds, and the obtained solution is purified RNA.

Note: The eluent needs to be added to the center of the purification column for complete absorption.To obtain a higher concentration of samples, the eluent volume can be reduced to 20 μL, but the amount of RNA eluted will be relatively reduced.When the room temperature is low, preheating the eluent at 37℃ for a while is helpful to the yield.In addition, the eluted solution was added back to the original purification column and eluted by centrifugation again, which could increase the yield by about 10-30%.Alternatively, a second elution with a new eluent after the first elution will yield approximately 15 to 40% of the original amount of RNA eluted.