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RNApure RNA Mini Kit

Product Number: RNK0301

Shipping and Storage

1. Therefore, transportation and storage are carried out at room temperature (15℃-25℃). The TRIpure can be transported at room temperature, and can be stored for a long time in a dark place at 4℃ after receipt. Storage at room temperature for 3 months does not affect the quality of use.
2. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly after use.

Component

Component	RNK0301 50rxns	RNK0302 100rxns
TRIpure Reagent (4℃, dark)	50mL	100mL
Buffer PE	16mL	32mL
	Add anhydrous ethanol as indicated on the bottle label before the first use	
Buffer RW	10mL	25mL
	Add anhydrous ethanol as indicated on the bottle label before the first use	
RNase-free H_2O	5mL	5mL
RNase free adsorption column RA	50	100
Collection tube (2mL)	50	100

This reagent kit (excluding low-temperature components) does not affect its effectiveness when stored at room temperature for 12 months.

Description

Improved guanidine isothiocyanate/phenol one-step method (TRIzol method) is used to lyse cells and inactivate RNAses. Total RNA is then selectively adsorbed onto the silica matrix membrane in a highly ionized salt state, followed by a series of rapid rinsing centrifugation steps to remove cell metabolites, proteins, and other impurities from the protein solution and rinse solution. Finally, low salt RNase free water is used to elute pure RNA from the silica matrix membrane.

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability.
2. Combining the advantages of good stability, high purity, and convenient and fast centrifugation column of guanidine isothiocyanate/phenol one-step reagent, it does not require isopropanol precipitation and ethanol washing process. RNA can be directly eluted from the centrifugation column to avoid the problem of excessive drying and difficult dissolution.
3. TRIpure Reagent corresponds to ThermoFisher/Invitrogen's TRIzol, which can effectively eliminate genomic contamination. The effect is consistent with the import.
4. Multiple rinsing and deproteinization processes result in higher purity of RNA extraction.
5. Effectively removed the content of 5S in total RNA and improved purity.

Note

1. This reagent kit exhibits excellent inhibition of RNA enzymes, and all centrifugation steps can be performed at room

temperature unless otherwise specified.

2. The TRIpure Reagent and Buffer PE contain irritating and harmful compounds. When operating, latex gloves should be worn to avoid contact with skin, eyes, and clothing. **If it comes into contact with the skin or eyes, rinse with plenty of water or physiological saline.**
3. Considering environmental protection issues, this reagent kit does not contain commonly used laboratory reagent chloroform, and you need to prepare your own chloroform before use. But if chloroform is really difficult to obtain, it can also be avoided.
4. Conventional agarose gel electrophoresis and denaturing gel electrophoresis can be used to analyze the quality of RNA. A good RNA product should show two distinct dominant ribosomal RNA bands after electrophoresis, namely~5Kb (28S) and~2Kb (18S), with a band brightness ratio of approximately 2:1.Sometimes~0.1kb and 0.3Kb (5S, tRNA) bands can also be seen. But sometimes it is normal to see 4-5 bands according to different species, such as certain plant tissues. If the precursor of RNA is immature or uneven nuclear RNA or small nuclear RNA is extracted, discontinuous high molecular weight bands between 7Kb and 15Kb may also be seen.
5. After adding TRIpure Reagent homogenate and before adding chloroform, the sample can be stored at -60℃ -70℃ for more than one month.
6. If extracting bacterial RNA, it is recommended to use the EASYspin Plus Bacterial Fast RNA Kit (RNK4302).

Protocol(Please read the note before the experiment)

Please add the specified amount of anhydrous ethanol to the Buffer RW bottle and the Buffer PE bottle before first use!

1. Homogenization treatment

1.1. Tissue

Grind the tissue in liquid nitrogen, add 1ml of TRIpure to every 50-100mg of tissue, and homogenize. The volume of the tissue sample cannot exceed 10% of the TRIpure volume.

1.2. Monolayer grown cells

Add 1ml of the TRIpure directly to the culture plate with a diameter of 3.5 cm to dissolve cells, and gently blow with a pipette to mix. Determine the required amount of the TRIpure based on the area of the culture plate rather than the number of cells (add 1ml per 10cm2). In general, in a regular sized cell culture bottle, add 1ml of the TRIpure and quickly shake it gently to fully contact all cells at the bottom of the bottle to lyse cells and inactivate RNA enzymes. Gently blow and mix with a pipette. When the amount of the TRIpure is insufficient, it can lead to DNA contamination in the extracted RNA.

Note: Cells cultured on the wall often cannot completely detach from the culture bottle (dish), which does not mean incomplete lysis. At this point, the cell membrane has actually completely ruptured and released all RNA. Continue with the procedure.

1.3. Suspended growing cells

Precipitate cells by centrifugation and carefully discard the supernatant. Every 5-10×106 animal cells, plant cells plus 1ml of the TRIpure. Use a pipette to repeatedly blow and break cells in the TRIpure reagents. Avoid washing cells before adding the TRIpure, as it may increase the likelihood of mRNA degradation.

2. Shake the homogenate sample vigorously and mix well. Incubate at 15-30°C for 5 minutes to completely decompose the nucleosomes.
3. Optional steps: Centrifuge at 12000 rpm for 10 minutes at 4°C, carefully remove the supernatant and transfer it into a new RNase free centrifuge tube. When the sample is rich in protein, fat, polysaccharides, or extracellular substances such as muscle, adipose tissue, or plant tuber parts, an additional separation step may be required.After homogenization, centrifuge at 12000 rpm for 10 minutes at 2-8°C to remove insoluble substances from the homogenate. The remaining precipitate contains extracellular membrane, polysaccharides, and high molecular weight DNA, while the upper layer of hyperplankton contains RNA.
4. Add 0.2 ml of chloroform to every 1ml of RL. Cover the sample tube tightly, shake vigorously for 15 seconds, and incubate it at room temperature for 5 minutes.

Note: If chloroform is not available, you can purchase Adlai's chloroform replacement reagent.

5. Centrifuge at 4℃ at 12000 rpm for 10 minutes, and the sample will be divided into three layers: the lower organic phase, the

middle layer, and the upper colorless aqueous phase. RNA is present in the aqueous phase. The capacity of the water phase layer is approximately 50% of the added TRIpure volume. Carefully transfer the water phase into a new tube (do not touch the middle layer) and record the water phase volume.

6. Add half the volume of the aqueous phase, which is 0.5 times the volume of anhydrous ethanol, and mix well (precipitation may occur at this time). The obtained solution and possible precipitates are transferred together into the adsorption column RA (the adsorption column is placed inside the collection tube. If it is not possible to add all the solution and mixture to the adsorption column RA at once, please transfer them into the adsorption column RA twice.) Centrifuge at 12000 rpm for 30 seconds, discard the waste liquid, and reinstall the adsorption column back into the collection tube.
7. Add 500μL of Buffer PE **(please check if anhydrous ethanol has been added first!)**, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
8. Add 500μL of Buffer RW **(please check if anhydrous ethanol has been added first!)**, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
9. Repeat step 8 once.
10. Return the adsorption column RA to the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove the Buffer RW as much as possible to prevent residual ethanol from inhibiting downstream reactions in the Buffer RW.
11. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 50-80μl of RNase free water to **the middle of the adsorption membrane** according to the expected RNA production. Leave at room temperature for 2 minutes and centrifuge at 12000rpm for 1 minute. If more RNA is needed, the obtained solution can be re added to the centrifuge adsorption column and centrifuged for 1 minute.

Note: The recommended elution volume is not less than 30μL, as a small volume can affect the efficiency of nucleic acid recovery.

The following steps can help increase the concentration of RNA products: RNase free H_2O is preheated at 80-90℃ before elution; Add the first eluent back to the adsorption column for a second elution.