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RNAliquid RNA Blood/Serum/Plasma Kit

Product Number: RNK2302

Shipping and Storage

1. Therefore, transportation and storage are carried out at room temperature (15℃ -25℃). Buffer RLS 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 in a timely manner after use.

Components

Component	Storage	RNK2302 50 Preps
Buffer RLS	4℃,protect from light	50 ml
Buffer RE	RT	25ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
RNase free adsorption column RA	RT	50 个
Collection tube (2ml)	RT	50 个

Description

The improved one-step method of guanidine isothiocyanate/phenol (TRIzol LS method for liquid samples) lyses cells and inactivates RNA enzymes. Then, total RNA is selectively adsorbed onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer RE and Buffer RW remove impurities such as cell metabolites and proteins, and finally, low salt RNase free water elutes 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, RNA can be directly eluted from the centrifugation column without the need for isopropanol precipitation and ethanol washing process, avoiding the problem of excessive drying and difficult dissolution.
3. The unique Buffer RLS formula can directly lyse whole blood without the need to first lyse and remove red blood cells.
4. Multiple rinses to remove proteins result in higher purity of extracted RNA.
5. Effectively removed the content of 5S in total RNA and improved purity.

Note

1. **Before the first use, please add the specified amount of ethanol to the Buffer RW bottle. After adding, please mark the added ethanol with a tick in a timely manner to avoid adding it multiple times!**
2. This reagent kit exhibits excellent inhibition of RNA enzymes, and all centrifugation steps can be performed at room temperature unless otherwise specified.
3. Buffer RLS and Buffer RE contain irritating and harmful compounds. When operating, latex gloves should be **worn to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.**
4. Considering environmental protection issues, this reagent kit does not contain commonly used laboratory reagent chloroform. Before use, it is necessary to prepare chloroform yourself.

5. 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 depending on different species, such as certain plant tissues. If RNA precursors are not mature or uneven nuclear RNA or small nuclear RNA is extracted, discontinuous high molecular weight bands between 7Kb and 15Kb may also be seen.
6. When testing the OD260/OD280 absorbance ratio, if RNA samples need to be diluted, TE (pH 8) should be used. If diluted with water and tested, due to the low water ion strength and pH value, OD280 will increase, resulting in a decrease in the ratio.
7. After adding buffer RLS homogenate and before adding chloroform, the sample can be stored at -60℃ -70℃ for more than one month.
8. If extracting bacterial RNA, it is recommended to use EASYspin Bacterial Fast RNA Kit (RNK0801) and REASYspin Plus Bacterial Fast RNA Kit (RNK4302).

Protocol(Please read the precautions before the experiment)

Tip: Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle!

1. Take 0.25 ml of blood (or serum, plasma, cerebrospinal fluid, etc.) and add 0.75 ml of Buffer RLS. Use a sampling gun to blow the liquid sample several times to help lyse the cells in the sample. The final volume ratio between Buffer RLS and liquid samples is always 3:1.
2. Shake the sample vigorously and mix well, then let it stand at room temperature for 5 minutes to completely decompose the ribosomes.
3. Add 0.2 ml of chloroform, vigorously shake for 15 seconds, and let it stand at room temperature for 2 minutes.
4. Centrifuge at 4℃ 13000 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 aqueous phase layer is approximately 60% of the volume of added RL S. Transfer the aqueous phase to a new tube for the next step of operation.
5. Add 0.5 times the volume of anhydrous ethanol, invert and mix well (precipitation may occur at this time). The obtained solution and possible precipitate are transferred together into the adsorption column RA (the adsorption column is nested inside the collection tube).
6. Centrifuge at 13000 rpm for 30 seconds at room temperature (all steps below are at room temperature), discard the waste liquid, and reinstall the adsorption column into the recovery manifold.
7. Add 500μl Buffer RE, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
8. Add 500μl Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 15 seconds, and discard the waste liquid.
9. Repeat step 8.
10. Return the adsorption column RA to the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
11. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50μl of RNase free water to the middle of the adsorption membrane according to the expected RNA production (heating in a 65-70 ℃ water bath beforehand is more effective). Let it stand at room temperature for 2 minutes and centrifuge at 13000 rpm 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, or an additional 30μl of RNase free water can be added and centrifuged for 1 minute, and the two elutions can be combined.

    If a high RNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μl. If the volume is too small, it will reduce RNA elution efficiency and RNA production.