MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

RNApure RNA Mini Kit

Product Number: RNK0302

Shipping and Storage

1. Therefore, transportation and storage are carried out at room temperature (15℃-25℃). The Buffer RL 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.
3. This reagent kit is stored at room temperature for 12 months without affecting its effectiveness.

Component

Component	RNK0302 50rxns
Buffer RL (4℃, dark)	50 ml
Buffer PE	16 ml
Buffer RW	10 ml
RNase-free H_2O	10 ml
RNase free adsorption column RA	50
Collection tube (2ml)	50

Description

The improved guanidine isothiocyanate/phenol one-step method (TRIzol method) cleaves cells and inactivates RNA enzymes. Then, total RNA is selectively adsorbed on the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, impurities such as cell metabolites and proteins are removed from the membrane using deproteinized and rinsed solutions. Finally, pure RNA is eluted from the silica matrix membrane using low salt RNase free water.

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. Unique Buffer RL formula that effectively eliminates genomic contamination.
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. **Before the first use, please add the specified amount of ethanol to the Buffer RW bottle and the Buffer PE bottle. After adding, please mark with a tick that ethanol has been added 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. The Buffer RL 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.**

4. 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.
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 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.
6. When testing the OD_{260}/OD_{280} absorbance ratio, TE (pH 8) should be used to dilute the RNA sample. If diluted with water and tested, due to the low water ion strength and pH value, OD_{280} will increase, resulting in a decrease in the ratio.
7. After adding Buffer RL 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 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 Buffer RL to every 50-100mg of tissue, and homogenize. The volume of the tissue sample cannot exceed 10% of the Buffer RL volume.

1.2. Monolayer grown cells

Add 1ml of the Buffer RL 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 Buffer RL based on the area of the culture plate rather than the number of cells (add 1ml per $10cm^2$). In general, in a regular sized cell culture bottle, add 1ml of the Buffer RL 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 Buffer RL is insufficient, it can lead to DNA contamination in the extracted RNA.

1.3. Suspended growing cells

Precipitate cells by centrifugation and carefully discard the supernatant. Every $5\text{-}10\times10^6$ animal cells, plant cells plus 1ml of the Buffer RL. Use a pipette to repeatedly blow and break cells in the Buffer RL reagents. Avoid washing cells before adding the Buffer RL, 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 cannot be obtained, the layering step of adding chloroform can also be omitted (steps 4 and 5 are omitted), and 0.5 times the volume of anhydrous ethanol is added to the supernatant of the pyrolysis homogenate obtained in step 3, followed by step 6. Layering without chloroform may result in slightly lower yield and purity.

5. Centrifuge at 4°C 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 RL 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 larger the elution volume, the higher the elution efficiency. If a higher 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, the RNA elution efficiency will be reduced and RNA production will be reduced.