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miEASY microRNA Kit

Product Number: RNK4601

Shipping and Storage

1. All solutions should be clear. If the ambient temperature is low, the solution may form precipitates and should not be used directly. It can be heated in a 37℃ water bath for a few minutes to restore clarity.
2. Inappropriate storage at low temperatures (4℃ or-20℃) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15℃-25℃).
3. Buffer WS 2/3 may precipitate crystals after using ethanol for a few days, which does not affect its use. Simply do not absorb the crystals and use the supernatant.
4. 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	RNK4601 50preps
Buffer LY	4℃,protect from light	50 ml
Buffer WS 1	RT	12 ml
Buffer WS 2/3	RT	10 ml
RNase-free H_2O	RT	10 ml
RNase free adsorption column RA and collection tube	RT	50

Description

In recent years, extensive research on RNA interference and regulatory small RNAs urgently requires a reagent kit that can effectively extract RNA of around 15-30 nucleotides in size (including SiRNA and miRNA). However, traditional RNA extraction methods such as silica gel membranes cannot effectively adsorb and recover small molecule RNA, and phenol/guanidine extraction and isopropanol or ethanol precipitation cannot effectively precipitate and recover small molecule RNA. For serum and plasma samples, it is even more difficult to extract due to its own characteristics. This reagent kit uses a unique Buffer LY to rapidly and directly lyse serum plasma RNA enzymes, with strong organic extraction to remove proteins and DNA. RNA, including small molecules, is adsorbed on a special silicon-based membrane in a centrifuge column under high concentration ethanol. Then, through a series of special Buffer WS rapid rinsing centrifugation steps, Buffer WS further removes impurities such as cell metabolites and proteins. Finally, low salt eluent elutes pure RNA from the silicon-based membrane.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. There is also no need for steps such as isopropanol or ethanol precipitation that are prone to losing small molecule RNA.
3. The special Buffer LY formula can handle more serum/plasma samples.
4. Multiple column washes ensure high purity and can be used for RNAi, RT-PCR, Northern blot, and various experiments.

Application

Suitable for purifying cell-free total RNA, including miRNA, from animal and human plasma and serum.

Note

For Research Use Only

1. Before the first use, please add the specified amount of ethanol to Buffer WS 1 bottle and Buffer WS 2/3 bottle. After adding, please mark with a check mark that ethanol has been added in a timely manner to avoid adding it multiple times!
2. Unless otherwise specified, all centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of 13000rpm, such as Eppendorf5415 ℃ or a similar centrifuge.
3. We need to provide our own ethanol and chloroform.
4. Buffer LY and Buffer WS 1 contain irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
5. RNA purity and concentration detection:

   In general, RNA production can be determined by measuring the OD260 value, and measuring the OD260/OD280 ratio can be one of the indicators to measure the degree of protein contamination. However, due to the extremely low RNA content in serum/plasma, which is already below the lower limit of the spectrophotometer measurement, it cannot be accurately measured. Therefore, purity or concentration cannot be determined by measuring the OD value or ratio, and can only be determined by downstream fluorescence quantitative RT-PCR. At the same time, the RNA in cell-free serum/plasma is mainly small RNA smaller than 100nt, so traditional electrophoresis detection of RNA integrity is not suitable for serum/plasma RNA.

Protocol

Tip: Before first use, please add the specified amount of ethanol to Buffer WS 1 bottle and Buffer WS 2/3 bottle!

1. Add 750μl Buffer LY to every 250μl of sample (serum, plasma), vortex shake or blow the liquid sample several times with a sampling gun to aid in lysis.

   For samples containing high pollutants, such as high protein and high blood lipid samples, the processing volume can be appropriately reduced, and the insufficient volume can be supplemented with RNase free H2O. The final volume ratio between Buffer LY and liquid samples is always 3:1. For example, 200μl sample+50μl RNase free H2O+750μl Buffer LY.
2. Shake the sample vigorously and mix well, incubate at 15-30℃ for 5 minutes.
3. Add 200μl of chloroform to every 750μl Buffer LY, vigorously shake for 15 seconds, and let it sit at room temperature for 2 minutes.
4. Centrifuge at 4℃ and 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 aqueous layer is approximately 70% of the volume of the buffer LY added.
5. Carefully take the supernatant (accurately calculate the volume) and transfer it into a new centrifuge tube. Add 1.5 times the volume of anhydrous ethanol (which must be at room temperature), vortex and mix well. At this point, precipitation may occur, but it does not affect the extraction process. Immediately blow and mix, do not centrifuge, and proceed to the next step immediately.
6. Add the mixture (less than 700μl each time, which can be added in two separate batches) to an adsorption column RA, centrifuge at 12000 rpm for 30-60 seconds, and discard the waste liquid.
7. Add 700μl Buffer WS 1 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
8. Add 500μl Buffer WS 2/3 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid. Add 500μl Buffer WS 2/3 and repeat.
9. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove the buffer WS as much as possible to avoid residual ethanol in the buffer WS inhibiting downstream reactions.
10. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-40μl of RNase free H2O to the middle of the adsorption membrane according to the expected RNA production (preheating in a 100 ℃ water bath is better), leave at room temperature for 1 minute, and centrifuge at 12000 rpm for 1 minute.

    Adding the eluent back to the adsorption column for repeated elution can increase yield and concentration (if high RNA concentration is required). If the concentration needs to be increased, the minimum elution volume can be as low as 15μl, but using a small volume for elution will reduce some yield.