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miEASY microRNA Blood/Serum/Plasma Kit

Product Number: RNK2401

Shipping and Storage

1. After adding anhydrous ethanol to Buffer WS1 and Buffer WS2/3, they can be stored at room temperature for one month. If they need to be stored for a longer period of time, please store them at 4℃. However, before use, they should be restored to room temperature first
2. Buffer WS2/3 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.
3. Transportation is carried out at room temperature without affecting the effectiveness of use
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	RNK2401 50Preps
Buffer LY	4°C light avoidance	50 ml
70% ethanol	RT	9ml RNase-free H_2O
Buffer WS1	RT	12 ml
Buffer WS2/3	RT	10 ml
RNase-free H_2O	RT	10 ml
Adsorption column RA and collection tube	RT	50
MicroRNA adsorption column MA 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 ethanol precipitation cannot effectively precipitate and recover small molecule RNA. For blood 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 whole blood (liquid samples) and inactivate cell RNA enzymes, with strong organic extraction to remove proteins and DNA. RNA, including small molecule RNA, is adsorbed onto a special silicon-based membrane in a centrifuge column. Then, through a series of rapid rinsing centrifugation steps, the rinse solution further removes impurities such as cell metabolites and proteins. Finally, low salt elution solution 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. It also does not require steps such as ethanol precipitation that are prone to losing small RNA molecules.
3. The unique Buffer LY formula can directly lyse whole blood without the need to first lyse and remove red blood cells.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.9~2.0 and minimal DNA residue, which can be used for RNAi, RT-PCR, Northern blot, and various experiments.

Application

Suitable for rapid extraction of miRNAs and other small RNAs from various whole blood/plasma/liquid samples

Note

1. Before the first use, please add the specified amount of ethanol to 70% ethanol, Buffer WS1 bottle, and Buffer WS2/3 bottle. After adding, please mark the added ethanol with a check mark in a timely manner to avoid multiple additions!
2. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm, such as Eppendorf5415C or a similar centrifuge.
3. You need to bring your own ethanol, chloroform, disposable syringe, and mortar.
4. Buffer LY and Buffer WS1 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. To prevent RNase pollution, attention should be paid to the following aspects:
   - 5.1. Regularly replace with new gloves. Because the skin often carries bacteria, it may lead to RNase contamination.
   - 5.2. Use RNase free plastic products and gun heads to avoid cross contamination.
   - 5.3. RNA will not be degraded by RNase when in Buffer LY. However, plastic and glassware without RNase should be used during the extraction and subsequent processing. Glassware can be baked at 150 ℃ for 4 hours, while plastic containers can be soaked in 0.5 M NaOH for 10 minutes, then thoroughly washed with water and sterilized to remove RNase.
   - 5.4. Prepare the solution using water without RNase. (Add water to a clean glass bottle, add DEPC to the final concentration of 0.1% (v/v), leave at 37℃ overnight, and sterilize under high pressure.)
6. RNA purity and concentration detection:
   - 6.1. Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5 × TBE electrophoresis buffer; 150v, 15min). Due to 70% -80% of RNA in cells being rRNA, very clear rRNA bands should be visible under UV after electrophoresis. Animal rRNAs are approximately 5kb and 2kb in size, equivalent to 28S and 18S rRNAs, respectively. The maximum rRNA brightness in animal RNA samples should be 1.5-2.0 times the brightness of the second largest rRNA, otherwise it indicates degradation of the RNA sample. The appearance of dispersed flakes or disappearance of bands indicates severe degradation of the sample.
   - 6.2. Purity: The OD260/OD280 ratio is an indicator of the degree of protein contamination. High quality RNA, OD260/OD280 reading (10mM Tris, pH 7.5) between 1.8-2.1. The OD260/OD280 reading is affected by the pH value of the solution used for measurement. Assuming that the OD260/OD280 readings for the same RNA sample are between 1.8-2.1 in a 10mM Tris, pH 7.5 solution, and between 1.5-1.9 in an aqueous solution, this does not mean that the RNA is impure.
   - 6.3. Concentration: Take a certain amount of RNA extract, dilute it n times with RNase free H_2O, set the spectrophotometer to zero with RNase free H_2O, take the diluent for OD260 and OD280 measurements, and calculate the RNA concentration according to the following formula: Final concentration (ng)/μl) =(OD260)×(dilution ratio n)×40.

Protocol

Tip: Before first use, please add the specified amount of ethanol to 70% ethanol, Buffer WS1 bottle, and Buffer WS2/3 bottle!

1. Add 0.75ml Buffer LY to every 0.25ml of liquid samples (serum, plasma, cerebrospinal fluid, etc.), and blow the liquid samples several times with a sampling gun to help lyse the cells in the samples. Add at least 0.75ml Buffer LY to every 5~10×10^6 cells. For samples containing high pollutants, such as whole blood samples, they can be diluted twice with sterilized water in a 1:1 ratio before extraction begins. The final volume ratio between Buffer LY and liquid samples is always 3:1.
2. Shake the sample vigorously and mix well. Incubate at 15-30℃ for 5 minutes to completely decompose the ribosomes.
3. Add 0.2ml of chloroform to every 0.75ml 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 without centrifugation, 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 WS1 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
8. Add 500µl Buffer WS2/3 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid. Add 500µl Buffer WS2/3 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 rinsing solution as much as possible to avoid residual ethanol in the rinsing solution inhibiting downstream reactions.
10. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50µl of RNase free H_2O 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.
11. If the expected RNA production is>30µg, add 30-50µl of RNase free H_2O and repeat step 10,Combine the two eluents, or use the first eluent to add back to the adsorption column and repeat step once (if high RNA concentration is required).

    The concentration of RNA elution solution after two washes is high, and the RNA production of the combined elution solution after two washes is 15-30% higher than the former, but the concentration is lower. Users can choose according to their needs.

Appendix: MicroRNA Enrichment Method

(Only microRNA is extracted, excluding other total RNA components>200mt.)

1. Follow the previous standard operating steps 1-5 until the upper clear is obtained.
2. Accurately estimate the volume of the supernatant, add an equal volume of 70% ethanol (please check if anhydrous ethanol has been added first!) (must be at room temperature), vortex or blow thoroughly, and do not centrifuge.
3. Add the mixture to an adsorption column RA, and centrifuge at 12000 rpm for 30-60 seconds to collect the filtered material. After transferring the filtered material from the collection tube to a new centrifuge tube, place the adsorption column back into the empty collection tube, add the remaining mixture, centrifuge, and collect the filtered material. Merge the two filtered materials and calculate the volume.

   At this point, the filtrate contains microRNA, and on the adsorption column is the total RNA removed from microRNA (excluding microRNA). If necessary, rinse according to the previous standard operating steps 7-10 to recover the total RNA removed from microRNA.
4. Accurately estimate the volume of the filtrate, add 0.65 times the volume of anhydrous ethanol (which must be at room temperature), vortex or blow thoroughly, and do not centrifuge.
5. Take a new set of microRNA adsorption column MA, add the mixture from the previous step (less than 700µl each time, can be added in two batches) to the microRNA adsorption column MA, and centrifuge at 12000 rpm for 30 seconds (the adsorption column is placed in a collection tube). Discard the waste liquid.
6. Follow the standard operating steps 7-10 to rinse and elute to obtain enriched microRNA.

   Note: Different experiments can choose different methods, such as Northern Blot or expression chip profiling, which can choose to extract total RNA including microRNA. The microRNAs extracted by enrichment methods may reduce the amplification background of some downstream experiments due to the removal of larger fragments of mRNA and rRNA. When the background is high or there is more non-specific amplification, enrichment methods can be used to extract microRNAs.