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miEASY microRNA Mini Kit

Product Number: RNK0501

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.

Component

Component	Storage	RNK0501 50Preps
Buffer LY/BD	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, while phenol/guanidine extraction and ethanol precipitation cannot effectively precipitate and recover small molecule RNA. This reagent kit uses a unique Buffer LY/BD, B-mercaptoethanol to rapidly lyse cells and inactivate cell RNA enzymes, with strong organic extraction to remove proteins and DNA. RNA, including small molecules, is adsorbed onto a special silicon-based membrane in a centrifuge column and then subjected to a series of rapid rinsing centrifugation steps. The rinsing solution further removes impurities such as cell metabolites and proteins, and finally, pure RNA is eluted from the silicon-based membrane using a low salt elution solution.

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. Fast and simple, the operation of a single sample can generally be completed within 30 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.9~2.0 and almost no DNA residue. It 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 cell tissues

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/BD 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, OD_{260}/OD_{280} reading (10mM Tris, pH 7.5) between 1.8-2.1. The OD_{260}/OD_{280} reading is affected by the pH value of the solution used for measurement. Assuming that the OD_{260}/OD_{280} 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 OD_{260} and OD_{280} measurements, and calculate the RNA concentration according to the following formula: Final concentration (ng)/μl) =(OD_{260})×(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. Tissue cultured cells
   - 1.1. Collect<10^7 suspended cells into a 15ml centrifuge tube. (For adherent cells, well plate culture and cell bottle culture can be directly lysed. After removing all remaining culture media as much as possible, 1ml of Buffer LY/BD can be directly added. Quickly and gently shake to allow Buffer LY/BD to fully contact all cells at the bottom of the bottle to lyse cells and inactivate RNA enzymes. Gently use a pipette to repeatedly blow and mix, and continue with step 3 of the operation.)
   - 1.2. Centrifuge at 13000rpm for 10 seconds (or 300g for 5 minutes) to allow the cells to precipitate. Completely aspirate and discard the supernatant, leaving behind cell clusters. Note that incomplete disposal of the supernatant will dilute Buffer LY/BD, leading to a decrease in yield and purity.
   - 1.3. Lightly flick the tube wall to completely loosen and resuspend the cell precipitate. Add 1ml Buffer LY/BD, vortex or blow, and thoroughly lyse and mix.
   - 1.4. Follow step 3 of the operation.
2. Animal tissues (such as mouse liver and brain)
   - 2.1. Fresh tissue is quickly cut into small pieces using a dissecting knife. According to the quality of the processed tissue, 1ml

of buffer LY/BD is added in a ratio of 50-100mg, and then thoroughly homogenized electrically or manually. Alternatively, after grinding the tissue into fine powder in liquid nitrogen, take an appropriate amount of tissue fine powder (about 50-100mg) and transfer it into a 1.5ml centrifuge tube containing 1ml Buffer LY/BD, vigorously blow and vortex to mix well.

2.2. Optional, generally not required: If the processing volume is large, there are obvious particles or insoluble substances, they are very viscous or the lysis is not sufficient, you can immediately use a disposable 5ml (about 0.9mm needle) syringe with a needle to pump the lysis 10 times or until a satisfactory homogenization result is obtained (or electric homogenization for 30 seconds), which can cut DNA to reduce viscosity and increase yield.

2.3. Follow step 3 of the operation.

3. Leave at room temperature for 5 minutes to fully separate the nucleic acid protein complex.
4. Add 200ul of chloroform and vigorously shake for 15 seconds.
5. Place at room temperature for 2-3 minutes and centrifuge at 13000rpm for 10 minutes.
6. Carefully take the supernatant (about 600μl) and transfer it into a new centrifuge tube. Add 1.5 times the volume of anhydrous ethanol (which must be at room temperature, usually 900μl) and vortex 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.
7. 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.
8. Add 700μl Buffer WS1 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
9. 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.
10. 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.
11. 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 12000rpm for 1 minute.
12. If the expected RNA production is>30mg, add 30-50μl of RNase free H_2O and repeat step 11 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 Methods

(Only microRNA was extracted, excluding other total RNA components>200nt.) Follow the previous standard operating steps 1-5 until the supernatant is obtained.

1. Accurately estimate the volume of the supernatant (about 600ul), 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.
2. Add the mixture to an adsorption column RA and centrifuge at 12000 rpm for 30-60 seconds (with the adsorption column placed in a collection tube) 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 (excluding microRNA) that has been removed from the microRNA. If necessary, rinse according to the previous standard operating steps 8-11 to recover the total RNA that has been removed from the microRNA.
3. 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.

4. Take a new set of microRNA adsorption column MA, add the mixture from the previous step (less than 700ul 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.
5. Follow the standard operating steps 8-11 to rinse and elute to obtain enriched microRNA.

   Note: Different experiments can choose different methods, such as Northem 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 IRNA. When the background is high or there is more non-specific amplification, enrichment methods can be used to extract microRNA.