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EASYspin fixed and embedded tissue microRNA Kit

Product Number: RNK3101

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 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.
4. Protease K is stored in a ready to use glycerol buffer and transported at room temperature. After receipt, store at room temperature not exceeding 25℃ for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years.
5. 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	RNK3101 50 Preps
Buffer PKD	RT	15 ml
Buffer RBC	RT	25 ml
Buffer RW	RT	10ml
Protease K solution	4℃	1 ml
RNase-free H_2O	RT	10 ml
Genomic DNA clearance column and collection tube	RT	50
RNA adsorption column RA and collection tube	RT	50

Description

This kit is designed for rapid extraction of RNA, including microRNA, from formalin fixed and paraffin embedded tissue samples. The unique Buffer PKD/Protease K rapidly cleaves cells to release RNA, and then the lysis mixture passes through a genomic DNA clearance column, where genomic DNA is cleared and RNA penetrates through filtration. After adjusting the binding conditions with ethanol, the filtered RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. Then, through a series of rapid rinsing centrifugation steps, the protein solution and Buffer RW are used to remove impurities such as cell metabolites and proteins. Finally, the pure RNA is eluted from the silica matrix membrane using low salt RNase free H2O. The obtained RNA can be used for experiments such as reverse transcription PCR and fluorescence quantitative PCR.

Features

1. No toxic reagents such as phenol and chloroform are used, and no steps such as ethanol precipitation are required.
2. Fast and simple, single sample RNA extraction can generally be completed within 1 hour.
3. The exclusive genome clearance column and formula of the reagent kit ensure effective clearance of genomic DNA residues. Generally, the obtained RNA does not require DNase digestion and can be directly used for reverse transcription PCR, fluorescence quantitative PCR, and other experiments.
4. Multiple column washes ensure high purity of RNA and can be directly used for various downstream experiments.

Application

Suitable for rapid extraction of RNA, including microRNA, from formalin fixed and paraffin embedded tissue samples. RNA

can be directly used for reverse transcription PCR and fluorescence quantitative PCR.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge that can reach a speed of 13000 rpm, such as Eppendorf 5415C or a similar centrifuge.
2. The sample processing capacity should never exceed the RA processing capacity of the genomic DNA clearance column and RNA adsorption column, otherwise it may cause DNA residue or a decrease in yield. There is a significant difference in RNA/DNA among different types of tissue cells, for example, the thymus and spleen have abundant DNA content, exceeding 5mg will exceed the column processing capacity. COS cells have abundant RNA content, exceeding 3x106 cells will exceed the column adsorption capacity. So when starting to explore the experimental conditions, if the DNA/RNA content of the sample is not clear, it is better to use less sample processing volume, such as not exceeding 210μm thick paraffin sections. In the future, the processing capacity will be increased or decreased based on the sample testing situation.
3. Buffer PKD and Buffer RBC 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.
4. To prevent RNase pollution, attention should be paid to the following aspects:
   4.1. Regularly replace with new gloves. Because the skin often carries bacteria, it may lead to RNase contamination.
   4.2. Use RNase free plastic products and gun heads to avoid cross contamination.
   4.3. During the RNA extraction process, plastic and glassware without RNase should be used. 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.
   4.4. 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.)
5. Regarding trace residues of DNA:
   Generally speaking, any total RNA extraction reagent cannot completely avoid trace residues of DNA during the extraction process (DNase digestion cannot achieve 100% residue free). Our company's EASYspin fixed and embedded tissue-microRNA Kit, due to its unique buffer system and genomic DNA clearance column technology, has cleared the vast majority of DNA and can be directly used for reverse transcription PCR and fluorescence quantitative PCR. In some special cases, such as residual DNA caused by excessive DNA content or strict mRNA expression analysis requiring fluorescence quantitative PCR, we suggest that when selecting templates and primers:
   5.1. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions.
   5.2. Select primer pairs with different sizes of amplified products on genomic DNA and cDNA.
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 the reaction between RNA and protein cross-linking during formalin fixation and embedding of paraffin embedded tissues, RNA breakage or degradation can generally occur. After electrophoresis, only a blurred and dispersed (smear) band pattern can be seen under UV, and as the storage time increases, the degradation fracture becomes more severe, and even a blurred band with a peak value of only about 100bp can be seen. This belongs to the normal situation of RNA extraction.
   6.2. Purity: The OD260/OD280 ratio is a reference indicator for measuring the degree of protein contamination. High quality RNA, OD260/OD280 reading (10mMTris, 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 water, adjust the spectrophotometer to zero with RNase free water, take the diluent for OD260 and OD280 measurements, and calculate

the RNA concentration according to the following formula: final concentration (ng/μl)=(OD260)×(dilution n)×40.

Protocol

Please add the specified amount of anhydrous ethanol to the Buffer RW bottle before first use!

1. Trim and remove excess embedded tissue outer paraffin, and slice into 10-20μm thick sections.
   The slice thickness must be ≥ 10μm, otherwise if it is too thin, it will shred the cells and cause microRNA loss during the dewaxing process.
2. Collect paraffin sections with a total thickness not exceeding 40μm into a 1.5-2ml centrifuge tube (such as 2 sections of 20μm and 4 sections of 10μm paraffin sections), or paraffin sections with a total thickness not exceeding 80μm into a 2ml centrifuge tube.
3. Add 1ml of 100% xylene and vortex for 10 seconds. Instantly centrifuge and immerse the entire tissue in xylene.
4. Melt paraffin in a 50℃ water bath for 3 minutes, centrifuge at the highest speed at 20-25℃ for 2 minutes, and collect tissue to the bottom of the tube.
5. Be careful to use a pipette to remove the supernatant xylene and be careful not to aspirate the sediment.
6. Add 1ml of anhydrous ethanol, vortex oscillate, centrifuge at maximum speed for 2 minutes, and carefully discard the supernatant ethanol.
7. Add 1ml of anhydrous ethanol, repeat step 6, and absorb all ethanol as much as possible.
8. Dry ethanol at room temperature or 37℃ for 10 minutes or until all ethanol evaporates.
   It is very important to completely dry ethanol, and trace amounts of ethanol residue can also lead to a decrease in RNA production.
9. Thoroughly resuspend the tissue precipitate in **140μl/230μl Buffer PKD** by blowing or vortex oscillation, briefly centrifuge to collect the liquid at the bottom of the tube, add 20μl of protease K, blow and mix well.
   The former refers to the amount added when the total thickness of the slice is ≤ 40μm, while the latter refers to the amount added when the total thickness of the slice is ≤ 80μm. The same goes for the rest.
10. Incubate at 55℃ for 15 minutes, then incubate at 80℃ for 15 minutes.
    After incubation at 55℃, the centrifuge tube can be taken out and placed at room temperature. After the water bath temperature rises to 80℃, it can be placed in the water bath and incubated accurately for 15 minutes. Even a 2-minute extension may lead to partial degradation of RNA.
11. Add **320μl/500μl Buffer RBC** and mix thoroughly to adjust the binding conditions.
12. Immediately add the mixture to a genomic DNA clearance column and centrifuge at 14000 rpm for 30 seconds (the clearance column is placed in a collection tube), while retaining the filtrate (RNA in the filtrate).
    It should be avoided to suck on potentially large, undigested flocs on the column to prevent clogging of the centrifuge column.
13. Add **1120μl/1750μl** anhydrous ethanol to the filtrate, immediately blow and mix well, do not centrifuge.
    If 1750μl of anhydrous ethanol is added, the filtrate should be transferred to a centrifuge tube with a capacity exceeding 3ml before being added.
14. Immediately add the mixture (less than 700μl each time, can be added multiple times) to an RNA adsorption column RA, centrifuge at 13000 rpm for 30 seconds (the adsorption column is placed in a collection tube), and discard the waste liquid.
15. 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. Add 500μl Buffer RW and repeat.
16. Put the adsorption column RA back into 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.
17. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30μl of RNase free water to the middle of the adsorption membrane according to the expected RNA yield (heating in a 70-90℃ water bath beforehand can increase yield). Leave at room temperature for 1 minute and centrifuge at 12000 rpm for 1 minute.
    The larger the elution volume, the higher the elution efficiency. If a higher RNA concentration is required, the elution

volume can be appropriately reduced. If a higher RNA concentration is required, the elution solution can be returned to the adsorption column RA and eluted again.