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EASYspin Plus bone tissue RNA

Product Number: RNK5401

Shipping and Storage

1. 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℃).
2. 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	RNK5401 50preps
Buffer CLB	RT	50 ml
PLANTaid	RT	5 ml
Buffer RLT Plus	RT	25 ml
Buffer RW1	RT	40 ml
Buffer RW	RT	10 ml
RNase-free H_2O	RT	10 ml
Genomic DNA clearance column and collection tube	RT	50
RNase free adsorption column RA and collection tube	RT	50

Description

Bone tissue is hard, bone cell density is low, and the peripheral matrix contains a large amount of mucins (proteoglycans) and RNA, which are difficult to separate and cannot be high-quality extracted using the traditional Trizol method. This reagent kit uses a unique phenol/chloroform free lysis solution and adds multiple components to remove bone tissue proteoglycans. Meanwhile, the unique genomic DNA clearance column technology can effectively remove gDNA residues, and the obtained RNA generally does not require DNase digestion and can be used for reverse transcription PCR, fluorescence quantitative PCR, and other experiments. The unique Buffer CLB and Buffer RLT Plus rapidly lyse cells and inactivate cell RNA enzymes, centrifuge precipitate to remove polysaccharides and secondary metabolites, then lyse the mixture and regulate RNA binding with ethanol to adsorb onto the genomic DNA clearance column. RNA is selectively washed and filtered, and residual DNA adsorbed on the genomic DNA clearance column cannot be washed off and discarded along with the column to remove DNA. After adjusting the binding conditions with ethanol, the filtered RNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer RW1 and Buffer RW remove impurities such as cell metabolites and proteins. Finally, low salt RNase free H_2O washes the pure RNA off the silica matrix membrane.

Features

1. No toxic reagents such as phenol and chloroform are used, and no steps such as ethanol precipitation are required.
2. Simplicity, single sample operation can generally be completed within 35 minutes, making it the simplest and fastest reagent kit in the world.
3. The independently developed genomic DNA clearance column technology can effectively remove gDNA residues, and the obtained RNA generally does not require DNase digestion and can be used for experiments such as reverse transcription PCR and fluorescence quantitative PCR.
4. Widely adaptable, it can extract various bone tissues, including mineralized bone tissue.
5. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.9~2.2 and almost no DNA residue. It can be used for RT-PCR, Northem blot, second-generation sequencing, and various experiments.

Application

Suitable for rapid extraction of total RNA from bone tissue cells, unique genomic DNA clearance column technology can effectively remove visible gDNA residues on electrophoresis. RNA can be used for reverse transcription PCR, fluorescence quantitative PCR, etc.

Note

1. All centrifugation steps can be completed at room temperature (centrifugation at 4℃ is also possible), using a traditional desktop centrifuge with a speed of up to 13000rpm, such as Eppendorf5415C or a similar centrifuge.
2. You need to bring your own ethanol, mortar, or other suitable bone tissue crushing device.
3. The sample processing capacity should never exceed the processing capacity of the genome clearance column DA and RNA adsorption column RA, otherwise it may cause DNA residue or yield reduction. When starting to explore the experimental conditions, if the DNA/RNA content of the sample is unclear, a smaller sample processing amount can be used. In the future, the processing amount can be increased or decreased according to the sample testing situation.
4. Buffer CLB, Buffer RLT Plus, and Buffer RW1 contain irritating compounds. Wear latex gloves 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. 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 EASYspin Plus RNA extraction product adopts our unique buffer system and genomic DNA clearance column technology, and the vast majority of DNA has been cleared without DNase digestion. It 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.

   5.3. Treat the RNA extract with DNaseI of RNase free. This reagent kit can also be used for RNA cleaning after DNaseI treatment. Please contact us for specific operating instructions.

   5.4. Before rinsing buffer RW1, perform DNaseI column digestion directly on the adsorption column RA. Before purchasing the DNase Digestion Kit (RNK3401), you can first request specific operating instructions.

Protocol

Tip: 1) Before first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle!

2)Take 1ml of Buffer CLB into a centrifuge tube (if there is precipitation or precipitation of Buffer CLB, it should be dissolved again in a 65℃ water bath), add 100μl of PLANTAid to Buffer CLB, mix upside down, and preheat in a 65℃ water bath. Multiple samples are prepared by scaling up in proportion.

1. Liquid nitrogen grinding method:

1.1. After clamping the broken bone tissue with bone forceps, place it in a mortar, add liquid ammonia and repeatedly grind it into fine powder. Pay attention to continuously adding and storing liquid ammonia after it evaporates.

1.2. Transfer 100mg of fine powder and add it to a preheated Buffer CLB (already added with PLANTAID) centrifuge tube. Immediately vortex vigorously for 30-60 seconds or use a suction head to blow and mix until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), which can shear DNA, reduce viscosity, and increase yield.

1.3. Immediately follow step 3 of the operation steps.

2. Other methods of bone tissue fragmentation:

2.1. Take 100mg of bone tissue and add 1ml of preheated Buffer CLB (already with PLANTAID) high-speed homogenizer to crush the homogenate. Alternatively, take 100mg of frozen and embedded bone slices crushed in liquid nitrogen and add

them to Buffer CLB (with added PLANTAID) to crush the homogenate.

2.2. Immediately follow step 3 of the operation steps.

3. Put it back into a 65℃ water bath for a short time (10 minutes), occasionally inverting 1-2 times in the middle to aid in cracking.
4. Centrifuge the cracked product at 13000rpm for 10 minutes to precipitate the non cracked fragments.
5. Take the supernatant of the lysate (more supernatant can be taken without exceeding the capacity of the genomic DNA scavenging column, which can increase yield) and transfer it to a new centrifuge tube. Add half the volume of anhydrous ethanol (0.5 volume) to the supernatant. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well without centrifugation.

   If there is floating material on the surface of the upper clear, use a suction head to pick up the liquid below.
6. Add the mixture (less than 720μl each time, which can be added in two separate batches) to a genome clearance column, centrifuge at 13000rpm for 2 minutes, and discard the waste liquid.

   Ensure that all the liquid is filtered through after centrifugation and there is no residue on the membrane. If necessary, increase the centrifugation force and time.
7. Place the genomic DNA clearance column in a clean 2ml centrifuge tube (without RNA free or DEPC treatment, usually a clean new centrifuge tube is sufficient. Alternatively, use a new clean collection tube that comes with the RNA adsorption column), add 500μl Buffer RLT Plus to the genomic DNA clearance column, centrifuge at 13000 rpm for 30 seconds, collect the filtrate (RNA in the filtrate), use a micropipette to accurately estimate the volume of the filtrate (usually around 450-500μl, the volume lost during filtration should be subtracted), and add 0.5 times the volume of anhydrous ethanol. Precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix, and do not centrifuge.
8. Immediately add the mixture (less than 720μl each time, it can be added in two separate batches) to an adsorption column RA, (the adsorption column is placed in a collection tube) centrifuge at 13000rpm for 2 minutes, and discard the waste liquid.

   Ensure that all the liquid is filtered through after centrifugation and there is no residue on the membrane. If necessary, increase the centrifugation force and time.
9. Add 700μl Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
10. Add 500μl of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid. Add 500μl Buffer RW and repeat.
11. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer RW as much as possible to avoid residual ethanol in Buffer RW inhibiting downstream reactions.
12. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 30-50μl 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 12000rpm for 1 minute.
13. If the expected RNA production is greater than 30μg, add 30-50μl of RNase free water and repeat step 12 combine the two washes, or use the first wash 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.