MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Allprep RNA-DNA-miRNA-protein Extract Kit

Product Number: RNK4701

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. 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	RNK4701 50Preps
Buffer RLT Plus	RT	50 ml
Buffer WS1	RT	12 ml
Buffer WS 2/3	RT	10 ml
RNase-free H_2O	RT	10 ml
Buffer IR	RT	25 ml
Buffer WB	RT	15ml
Buffer APP	RT	60 ml
Buffer EB	RT	10 ml
Genomic DNA adsorption column DA and collection tube	RT	50
RNA adsorption column RA and collection tube	RT	50

Description

This kit is designed to rapidly extract and isolate genomic DNA, as well as total RNA and Proten, including miRNA, from the same animal cell or tissue sample. The unique Buffer RLT Plus rapidly cleaves and inactivates cellular RNA/DNA enzymes, and then cleaves the mixture of RNA/miRNA/DNA/Protein while passing through a genomic DNA adsorption column, where genomic DNA is adsorbed and miRNA/RNA/Protein penetrates and filters through. The genomic DNA on the DNA adsorption column undergoes a series of rinsing centrifugation to obtain pure genomic DNA. After adjusting the binding conditions with ethanol, the filtered miRNA/RNA selectively adsorbs onto the miRNA/RNA adsorption column in a highly dissociated salt state, and then obtains pure miRNA/RNA through a series of rapid rinsing centrifugal elution. The filtrate was selectively precipitated to obtain Protein. On the basis of phenol free and chloroform free DNA/RNA rapid extraction technology, combined with exclusive separation technology, the miRNA/RNA/genomic DNA obtained simultaneously has high purity and does not interfere with each other. The obtained miRNA/RNA does not require DNase digestion and can be directly used for experiments such as reverse transcription PCR and fluorescence quantitative PCR. Genomic DNA can also be directly used for various downstream experiments such as Southern, enzyme digestion, PCR, etc.

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, the separation of miRNA/RNA/genomic DNA/Protein from a single sample can generally be completed within 1 hour.
3. The exclusive adsorption column and formula ensure effective removal of genomic DNA residues. Generally, the miRNA/RNA obtained 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 miRNA/RNA/genomic DNA, which can be directly used in various downstream experiments.

Application

Suitable for rapid extraction and separation of genomic DNA and total RNA and Protein containing miRNA from the same animal cell or tissue sample (such as purchasing additional miRNA adsorption columns and matching solutions, and can also separate and extract total RNA and miRNA from the same sample to enrich miRNA), without the need for DNase digestion, 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 with a rotational speed of 13000rpm, such as Eppendorf5415 ℃ or a similar centrifuge.
2. The sample processing capacity should never exceed the processing capacity of the genomic adsorption column DA and RNA adsorption column RA, otherwise it may cause DNA residue or a decrease in production. There is a significant difference in RNA/DNA among different types of tissue cells, for example, the thymus is rich in DNA content, exceeding 5mg will exceed the column processing capacity. COS cells have abundant RNA content, exceeding 3×10^6 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 a smaller sample processing volume, such as cells not exceeding $3\text{-}4\times10^6$ and tissues not exceeding 10mg. In the future, the processing capacity will be increased or decreased based on the sample testing situation.
3. Buffer RLT Plus, Buffer IR, Buffer WS1, and Buffer WS 2/3 contain irritating compounds. When operating, wear latex gloves 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. If this reagent kit is needed for the extraction of DNA/miRNA/RNA from plant samples, especially difficult samples with abundant secondary metabolites of polysaccharides and polyphenols, please consult technical personnel as other reagents may be required.
5. If additional miRNA adsorption columns and matching solutions are purchased, the total RNA and miRNA of the same sample can also be separated and extracted to enrich miRNA. Please consult us.
6. 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 Allprep tissue/cell miRNA/DNA/Protein extraction kit, due to its unique buffer system and genomic DNA separation and clearance technology, has cleared the vast majority of DNA and does not require 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:

   6.1. Select primers that cross introns to pass through the connecting regions in mRNA, so that DNA cannot serve as a template for amplification reactions.

   6.2. Select primer pairs with different sizes of amplified products on genomic DNA and cDNA.

Protocol

Tip: Before first use, please add the specified amount of anhydrous ethanol to Buffer WB and 70% ethanol bottles!

1. Tissue cultured cells

1.1. Collect<10^7 suspended cells into a 1.5ml centrifuge tube. For adherent cells, well plate culture can directly lyse them. Cell bottle culture should first be digested with trypsin and then collected by blowing.

1.2. Centrifuge at 13000rpm for 10 seconds (or 300×g 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 the Buffer RLT Plus, leading to a decrease in yield and purity.

1.3. Lightly flick the tube wall to **completely loosen the cell** precipitate and resuspend. Add 350µl (<5×10^6 cells) or 600µl (5×10^6-1×10^7 cells) of Buffer RLT Plus, blow and mix well, then vigorously shake by hand for 20 seconds to fully lyse

1.4. Homogenization: (When dealing with very small cell volumes<1×10^5, it is generally not necessary, vortex oscillation for one minute to homogenize). Using a disposable 1ml syringe with a blunt needle (paired with a 0.9mm needle), extract the lysate 5-10 times or until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds). This can cut DNA, reduce viscosity, prevent clogging of the column, and increase yield.

1.5. Add all the cracked mixture or homogenate mixture onto the DNA adsorption column (the adsorption column is placed in the collection tube).

1.6. Follow the step 3 of the protocol

2. Animal tissues (such as mouse liver and brain)

2.1. **Electric homogenization:** Quickly cut fresh tissue into small pieces using a dissecting knife, add 350µl (<20mg tissue) or 600µl (20-30mg tissue) of Buffer RLT Plus, and thoroughly homogenize by electric force for 20-40 seconds.

2.2. **Liquid nitrogen grinding+homogenization:** After grinding the tissue into fine powder in liquid helium, take an appropriate amount of tissue fine powder (20mg/30mg) and transfer it into a 1.5m centrifuge tube containing 350µl/600µl tissue buffer RLT Plus. Shake vigorously by hand for 20 seconds to fully decompose. Using a disposable 1ml syringe with a blunt needle (paired with a 0.9mm needle), the lysate can be aspirated 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds). This can cut DNA, reduce viscosity, prevent clogging of the column, and increase yield.

2.3. Centrifuge the lysate at 13000rpm for 3 minutes after homogenization, precipitate any fragments or insoluble substances that may be difficult to lyse, and add all the supernatant of the lysate to the DNA adsorption column (the adsorption column is placed in a collection tube).

2.4. Follow the step 3 of the protocol

3. Centrifuge at 13000rpm for 30 seconds and retain the filtrate (miRNA/RNA/Protein in the filtrate). The DNA adsorption column (with genomic DNA adsorbed on the membrane) can be stored at 4 ℃ for a short period of time for future use.

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.

The following steps are for extracting RNA:

4. Use a micropipette to accurately estimate the volume of the filtrate (around 350µl or 600µl, the loss volume during filtration should be subtracted). Add 1.25 times the volume of anhydrous ethanol. Precipitation may occur at this time, but it does not affect the extraction process. **Immediately blow and mix well without centrifugation.**

5. Immediately add the mixture (less than 700µl each time, which can be added in two separate batches) to an RNA adsorption column RA, and centrifuge at 13000rpm for 30 seconds (the adsorption column is placed in a collection tube), **retaining the filtrate for protein extraction.**

The filtrate contains Protein. Please transfer it to a centrifuge tube of appropriate size (at least twice the volume of the filtrate) and retain it for Protein extraction starting from step 17. The collection tube of the DNA adsorption column containing filtered liquid and ethanol complex (the empty collection tube left after the mixture is transferred to the RNA adsorption column) needs to be retained. The DNA adsorption column should be returned to this collection tube and kept at 4 ℃ for use in genomic DNA extraction starting from step 11.

6. Add 700µl Buffer WS1 (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

7. 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.

8. Put the adsorption column RA back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

9. 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 production. Leave at room temperature for 1 minute and

centrifuge at 12000 rpm for 1 minute.

10. If the obtained RNA can be immediately used for downstream reactions or stored at low temperatures as soon as possible.

The following steps are for extracting DNA:

11. Add 500μl Buffer IR to the DNA adsorption column in step 3, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
12. Add 700μl Buffer WB **(please check if anhydrous ethanol has been added first!)**, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
13. Add 500μl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
14. Put the DNA adsorption column back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
15. Take out the DNA adsorption column and place it in a clean centrifuge tube. Add 100μl Buffer EB to **the middle of the adsorption membrane** (Buffer EB is better preheated in a 65-70 ℃ water bath), leave at room temperature for 3-5 minutes, and centrifuge at 12000 rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 12000 rpm for 1 minute.

    The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50μl. If the volume is too small, the DNA elution efficiency will be reduced, reducing DNA production.
16. DNA can be stored at 2-8 ℃, and if it needs to be stored for a long time, it can be placed at -20 ℃.

The following steps are for extracting Protein:

17. Add an equal volume of Buffer APP to the filtrate from step 5, mix well with vortex oscillation, and allow to settle Protein at room temperature for 15 minutes.
18. Centrifuge at 13000rpm for 5-10 minutes, be careful to discard the supernatant. Add 0.5ml of 70% ethanol, invert and centrifuge for 1 minute. Discard the supernatant carefully, leaving behind protein precipitates. Use a pipette to remove any remaining liquid as much as possible.
19. Dry the precipitate at room temperature for 5-10 minutes, and be sure to let the ethanol evaporate completely to avoid affecting downstream experiments.
20. Dissolve the protein precipitate in 30-150μl of 1×protein sample buffer (bromophenol blue should not be added to the buffer if protein quantification is required) or other downstream test required buffer.

    Due to the strong denaturation effect of Buffer RLT Plus or different protein isoelectric points, protein dissolution may be difficult. Use a pipette to blow or change the pH value to help protein dissolution, and briefly centrifuge to obtain the supernatant for use. Alternatively, 5% SDS or 8M urea can be used to dissolve the protein precipitate and perform protein quantification.

    Note that if BCA protein quantification is required, it may be necessary to dilute 8M urea to 3M.
21. Dissolve and denature the protein at 95 ℃ for 5 minutes, return to room temperature, centrifuge at the highest speed for 1 minute, and take the supernatant for SDS-PAGE electrophoresis or Western blot tests.