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EASYall RNA/DNA Mini Kit

Product Number: RNK2901

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	RNK2901 20Preps	RNK2902 50Preps
Buffer RLT Plus	RT	20 ml	50 ml
Buffer RW1	RT	15 ml	40 ml
Buffer RW	RT	5 ml	10 ml
RNase-free H_2O	RT	10 ml	10 ml
70% ethanol	RT	4ml RNase-free H_2O	9ml RNase-free H_2O
Buffer IR	RT	10 ml	25 ml
Buffer WB	RT	15 ml	15m1
Buffer EB	RT	10 ml	10ml
Genomic DNA adsorption column DA and collection tube	RT	20	50
RNA adsorption column RA and collection tube	RT	20	50

Description

This kit is designed for rapid extraction and isolation of genomic DNA and total RNA from the same animal cell or tissue sample. The unique Buffer RLT Plus rapidly cleaves cells and inactivates cellular RNA/DNA enzymes, and then cleaves the mixture of DNA/RNA while passing through a genomic DNA adsorption column. The genomic DNA is adsorbed while RNA penetrates and filters through the DNA adsorption column. After a series of rinsing centrifugation, the genomic DNA is eluted to obtain pure genomic DNA. After adjusting the binding conditions with ethanol, the filtered RNA selectively adsorbs onto the RNA adsorption column in a highly dissociated salt state, and then obtains pure RNA through a series of rapid rinsing centrifugation elution. The RNA/genomic DNA obtained simultaneously using phenol free and DNA like rapid extraction technology combined with exclusive separation technology has high purity and does not interfere with each other. The obtained 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, simple, and single sample RNA/genomic DNA isolation and extraction operations can generally be completed within 40 minutes.
3. The exclusive adsorption column and formula of the reagent kit ensure effective removal 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/genomic DNA, which can be directly used in various downstream

experiments.

Application

Suitable for rapid extraction and isolation of genomic DNA and total RNA from the same animal cell or tissue sample, 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 traditional desktop centrifuges such as Eppendorf 5415℃ or similar centrifuges that can reach a speed of 13000rpm.
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 and spleen have abundant DNA content, exceeding 5mg will exceed the column processing capacity. COS cells have abundant RNA content, exceeding 3-4×10^6 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 amount, such as cells not exceeding 3-4×10^6 and tissues not exceeding 10mg. The processing capacity will be increased or decreased based on the sample testing situation.**
3. Buffer RLT Plus, Buffer IR, and deproteinized liquid RW1 contain irritating compounds. When operating, latex gloves should be worn 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. RNA will not be degraded by RNase in Buffer RLT Plus. 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.
   - 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 EASY all RNA/DNA Mini 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:
   - 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 RNA extract with DNaseI of RNase free to improve efficacy. 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 treatment directly on the adsorption column RA. Please contact us to request specific operating instructions.
6. RNA purity and concentration detection:
   - 6.1. Integrity: RNA can be detected by ordinary agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5xTBE 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 RNA sizes are approximately 5kb and 2kb, equivalent to

28S and 18S rRNA, 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 a reference indicator for measuring the degree of protein contamination. High quality RNA, OD260/0D280 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 H2O, 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(Please read the precautions before the experiment)

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

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 amounts of cells,<1×10^5 is generally not needed, vortex oscillation for one minute to homogenize). Using a disposable 1ml (with a 0.9mm needle) syringe with a blunt needle, vigorously tap the lysate more than 10 times or until satisfactory homogenization results are obtained (or electric homogenization for 30 seconds), DNA can be cut, viscosity can be reduced, column blockage can be prevented, and yield can be increased.

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. Immediately proceed to step 3 of the operation.

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 ammonia, take an appropriate amount of tissue fine powder (20mg/30mg) and transfer it into a 1.5ml centrifuge tube containing 350μl/600μl Buffer RLT Plus. Shake vigorously by hand for 20 seconds to fully crack. Using a disposable 1ml syringe with a blunt needle (paired with a 0.9mm needle), vigorously tap the lysate 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. Immediately proceed to step 3 of the operation.

3. Immediately centrifuge at 14000rpm for 60 seconds and retain the filtrate (RNA 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 (usually 350μl/600μl, the loss volume should be subtracted during filtration), add an equal volume of 70% ethanol (please check if anhydrous ethanol has been added first!), precipitation may occur at this time, but it does not affect the extraction process. Immediately blow and mix well, do not centrifuge.
5. Immediately add the mixture (less than 700μl each time, which can be added in two separate batches) to an RNA adsorption column RA (the adsorption column is placed in a collection tube) and centrifuge at 13000rpm for 30 seconds. Discard the waste liquid.

   The collection tube for the DNA adsorption column containing a mixture of filtered liquid and ethanol needs to be retained. The DNA adsorption column should be placed in the collection tube and kept at 4℃ for use in genomic DNA extraction starting from step 11.
6. Add 700μl Buffer RW1, let it stand at room temperature for 1 minute, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
7. Add 500μl of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid. Add 500μl Buffer RW 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 RW as much as possible to avoid residual ethanol in Buffer RW 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 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.
10. If the expected RNA production is>30ug, add 30-50μl of RNase free water and repeat step 9. 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.

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, it will reduce the DNA elution efficiency and 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℃.