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# EZ spin plus Mini RNA extraction kit

**Product Number: RNK3901**

## 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.
3. This reagent kit should be stored at room temperature for 12 months without affecting its effectiveness. Poly Carrier can be transported at room temperature and stored at 4℃ for one month. It can be stored for a long time at -20℃

## Components

| Component | Storage | RNK3901 50Preps |
|---|---|---|
| Buffer RLT Plus | RT | 25 ml |
| Buffer RW1 | RT | 40 ml |
| Buffer RW | RT | 10 ml |
| 70% ethanol | RT | 9 ml |
| RNase-free $H_2O$ | RT | 10 ml |
| Poly Carrier | -20℃ | 200 μl |
| Universal column and collection tube for micro DNA clearance/RNA adsorption | RT | 100 |
| Micro grinding pestle | RT | 3 |

## Description

This product is a specialized reagent kit for extracting trace amounts of RNA. Suitable for extracting total RNA from trace amounts of animal cells, micro cut tissues, and easily lysed animal tissues. The processing range is generally cells ($<10^6$) or tissues (<5mg). The exclusive DNA clearance/RNA adsorption universal column technology, combined with a special reagent formula that does not require DNA enzyme digestion, effectively removes gDNA residues. The obtained RNA has no DNA residue and can be directly used for downstream reverse transcription fluorescence quantitative PCR or high-throughput sequencing library construction experiments.

## 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 operation of a single sample can generally be completed within 10 minutes.
3. The exclusive development of DNA clearance/RNA adsorption universal column technology ensures effective removal of gDNA residues. The obtained RNA does not require DNase digestion and can be directly used for reverse transcription fluorescence quantitative PCR, high-throughput sequencing library construction and other experiments.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of up to 2.0-2.2 and minimal DNA residue. It can be used for RT-PCR, Northern blot, and various experiments.

## Note

1. All centrifugation steps are completed at room temperature.
2. The sample processing capacity should not exceed the capacity of the universal column for DNA clearance/RNA adsorption, otherwise it may cause DNA residue or a decrease in yield. If the cell processing capacity does not exceed 106 and the tissue does not exceed 5mg.

3. This reagent kit adopts the optimal design of a microcentrifuge column, theoretically capable of extracting at least 10 tissue cells.
4. Buffer RLT Plus and Buffer RW1 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. Our EASYspin Plus RNA extraction product adopts our unique buffer system and DNA clearance/RNA adsorption universal column/special reagent formula technology. The vast majority of DNA has been cleared and does not require DNase digestion. It can be directly used for reverse transcription fluorescence quantitative PCR. If downstream experiments are highly sensitive to trace amounts of DNA, DNase I can be used to further remove DNA contamination. Alternatively, during extraction, DNA enzyme digestion can be directly performed on a centrifuge column (RNK3401-DNase Digestion Kit)

## Protocol(Please read the notes before the experiment)

Tip: 1) Before the first use, please add the specified amount of anhydrous ethanol to the Buffer RW bottle and 70% ethanol bottle!

2) If the processed cell count is less than 5000 or the processed tissue count is less than 10ug, please add 4ul Poly Carrier to Buffer RLT Plus before homogenization

**1. Cultivate cells**

1.1.1. Adhering cells: do not require digestion. After thoroughly absorbing the culture liquid, directly add 350ul Buffer RLT Plus and repeatedly blow and lyse the cells. Add all the lysate mixture to the DNA clearance/RNA adsorption universal column (the universal column is placed in the collection tube) and directly proceed to operation step 3; For culture containers that are not convenient for direct lysis, cells can be scraped off with a cell scraper or collected into a 1.5ml centrifuge tube after trypsin digestion.

1.1.2. Suspended cells: Collect<$10^6$ suspended cells into a 1.5 ml centrifuge tube.

1.2. Centrifuge at 13000 rpm 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 the Buffer RLT Plus, leading to a decrease in yield and purity.

1.3. Gently tap the bottom of the centrifuge tube to loosen the cell precipitate, add 350ul Buffer RLT Plus, and use a pipette to repeatedly blow and thoroughly lyse (until no cell clusters are visible).

1.4. Add all the lysis mixture to the DNA clearance/RNA adsorption universal column (the universal column is placed in the collection tube).

1.5. Immediately proceed to step 3 of the operation.

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

2.1.1. Electric homogenization: Mix<5mg of tissue with 350ul Buffer RLT Plus and thoroughly homogenize for 20-40 seconds.

2.1.2. Grinding pestle+homogenization: In a 1.5 ml centrifuge tube, add 100ul Buffer RLT Plus and<5mg of tissue. Immediately grind the homogenization completely with a micro grinding pestle. Supplement the Buffer RLT Plus to 350ul.

2.1.3. Liquid nitrogen grinding+homogenization: After grinding the tissue into fine powder in liquid nitrogen, take an appropriate amount of tissue fine powder (<5mg) and transfer it into a 1.5mL centrifuge tube containing 350ul Buffer RLT Plus. Vortex for 20 seconds to fully decompose. Difficult to break samples can be homogenized by repeatedly blowing the mixture with a pipette or grinding with a pestle.

2.2. Add all the homogenate mixture onto the DNA clearance/RNA adsorption universal column (the universal column is placed in the collection tube).

2.3. Immediately proceed to step 3 of the operation.

3. Immediately centrifuge at 13000 rpm for 1 minute and retain the filtrate (RNA in the filtrate).
4. Use a micropipette to accurately estimate the volume of filtrate (usually 350ul), add an equal volume of 70% ethanol (please check if anhydrous ethanol has been added first!). At this time, precipitation may occur, but it does not affect the extraction

process. Immediately blow and mix well, do not centrifuge.

5. Immediately add the mixture to a new DNA clearance/RNA adsorption universal column, (the universal column is placed in a collection tube), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid.
6. Add 700ul Buffer RW1, let it stand at room temperature for 30 seconds, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
7. Add 500ul of Buffer RW (please check if anhydrous ethanol has been added first!), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid. Add 500ul Buffer RW and repeat.
8. Place the DNA clearance/RNA adsorption universal column 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.
9. Take out the universal column and place it in a clean 1.5ml centrifuge tube. Add 15-25ul of RNase free water to the middle of the adsorption membrane according to the expected RNA production (heating in a water bath at 80-100 ℃ beforehand can increase production). Leave at room temperature for 1 minute and centrifuge at 13000 rpm for 1 minute to obtain the RNA solution.

**Reducing the elution volume can increase RNA concentration, but RNA production will decrease, and users can choose according to their needs.**