Gene-Foci Biotechnologies

EASYspin Plus RNA Extraction Kit

- Catalog No. GF2705
- User's Manual
- For Research Use Only
- In vitro Use Only

EASYspin RNA Extraction Kit

Catalog No.：GF2705

Catalog No.	Preps
GF2705-50	50
GF2705-200	200

❖ APPLICATIONS

Ideal for rapid purification of genomic DNA-free total RNA from animal cells or tissues. The unique genomic DNA clearance columns effectively remove genomic DNA from RNA samples. No DNase digestion step is required. The purified total RNA can be directly used for reverse transcription and RT-PCR.

❖ Kit Contents And Storage Conditions

PCR Purification Kit	Storage Conditions	50 preps	200 preps
Lysis Buffer RLT Plus	Room Temp.	50 ml	200 ml
Buffer RW1	Room Temp.	40 ml	160 ml
Wash Buffer RW	Room Temp.	10 ml Add ethanol before first use	40 ml
RNase-free H_2O	Room Temp.	10 ml	40 ml
70% Ethanol	Room Temp.	9 ml RNase-free H_2O Add ethanol before first use	36 ml RNase-free H_2O
Genomic DNA columns DA and Collection Tubes	Room Temp.	50 Sets	200 Sets
RNase-free Columns RA and Collection Tubes	Room Temp.	50 Sets	200 Sets

❖ NOTES

1. This kit can be stored at room temperature for up to 12 months without showing any decrease in quality and yield
2. All buffers should be clear. Lower temperature may cause precipitation. If any precipitation forms, warm at 37C water bath to dissolve before use.
3. The Easyspin RNA Extraction kit should be stored at room temperature, store at 4°C or -20°C may cause chemical compound precipitation in buffers.
4. Recap the bottles **immediately** after use to avoid unexpected oxidation, evaporation and change of pH due to long term exposure to the air.

❖ INTRODUCTION

The innovative EASYspin plus system is ideal or rapid purification of genomic DNA-free total RNA from animal cells or tissues. The unique genomic DNA clearance columns effectively remove genomic DNA from RNA samples. No DNase digestion step is required. The purified total RNA can be directly used for reverse transcription and RT-PCR. The whole precess is phenol/chloroform-free. The unique lysis buffer immediately lyses biological samples and inactivates RNase and DNase. The lysate containing RNA is then passed through a genomic DNA clearance column, where genomic DNA binds to the column membrane, and RNA remains in the flow-through. Ethanol is added to the flow-through to provide appropriate binding conditions for RNA, and RNA selectively binds to the silica-membrane of the RNA column in the high-salt buffer. RNA is purified through a series of wash-spin steps to remove protein followed by elution of RNA from silica-membrane with RNase-free H_2O.

❖ HIGHLIGHTS

1. The unique DNA clearance column can effectively remove genomic DNA, and the purified RNA can be directly used for reverse transcription and RT-PCR without DNase digestion.

2. High quality silica membranes are used to ensure the yield and consistency between different batches.

3. Fast, and convenient. The whole RNA purification process from one sample can be done within 30 min**.**

4. Multiple washing steps guarantee high-quality RNA purification. The $OD_{260/280}$ of the RNA product is typically between 1.8-2.1.

❖ ATTENTION

1. All the steps should be performed at room temperature, use microcentrifuge such as Eppendorf 5415C or similar model that can handle 13,000 rpm or higher speed.

2. Materials and reagents to be supplied by the user: ethanol, 2-mercaptoethanol, single use syringes, mortar and pestle.

3. To obtain optimal DNA/RNA yield and purity, the amount of the processed sample must not exceed the maximum binding capacities of DA and RA columns. The DNA and RNA contents mary vary greatly between tissue/cell types. For example, DNA is enriched in the thymus and the spleen, and 5 mg of the tissue will be over the binding capacity of DA column. COS cells have high RNA content, more than $3x10^6$ cells will exceed the capacity of RA column. Use less starting materials when there is no information on their DNA/RNA contents.

We recommend starting with ≤ 3-4x106 cells or ≤ 10 mg tissues. Depending on DNA/RNA yield and purity, it may be possible to increase the sample amount in subsequent preparations.

4. Lysis buffer RLT and wash buffer RW1 contain irritating chemicals, wear gloves when handling. **Avoid direct contact with skin, eyes and clothes. If contaminated, rinse with large amount of water immediately.**

5. To prevent RNase contamination, the following precautions should be taken when handling RNA:

1). Change gloves frequently to avoid RNase contamination from the skin.

2). Use RNase-free plasticware and tips to avoid cross-contamination.

3). RNA will not be degraded in Buffer RLT Plus. But in the subsequent steps, RNase-free plasticware and glassware should be used. Glassware should be oven baked at 150°C for 4 hr. Plasticware can be treated with 0.5 M NaOH for 10 min, followed by thorough rinse with water and autoclave.

4). Use RNase-free, DEPC-treated water to prepare solutions (add DEPC to water at a final concentration of 0.1% (v/v), and leave at 37°C overnight and autoclave).

6. RNA detection:

Integrity of RNA: The integrity of the purified RNA can be detected by agarose gel electrophoresis (1.2% agarose gel; 0.5x TBE buffer). The ribosomal RNA (rRNA) should appear as sharp bands on the ethidium bromide-stained gel under UV. 28S rRNA bands should be present with the intensity approximately twice that of the 18S rRNA band. If the rRNA bands appear as a smear of smaller sized RNAs, it is likely that the RNA sample is degraded during preparation.

Purity of RNA: The ratio of OD_{260}/OD_{280} provides an estimate of the purity of

RNA with respect protein contamination. However, the OD_{260}/OD_{280} ratio is influenced considerably by pH. Lower pH results in a lower OD_{260}/OD_{280} ratio and reduced sensitivity to protein contamination. In 10mM Tris, pH7.5, pure RNA has a OD_{260}/OD_{280} ratio of 1.8-2.1. In water, the ratio is 1.5-1.9, and this does not mean the RNA is not pure.

Quantification of RNA: Dilute the RNA sample with RNase-free water, and measure the OD260 using a spectrophotometer which has been calibrated with RNase-free water. The concentration of RNA sample (ng/μl) is calculated using the following formula: OD_{260} x 40 x dilution factor.

❖ EASYSPIN PLUS RNA EXTRACTION KIT PROTOCOL

Please read "Attention" part before start.

Hints：

⇨ Before the first use, add the indicated amount of ethanol into buffer RW and 70% ethanol bottles, mix well, and mark the bottle with a check.

Procedure:

1. **Culture cells**
   a. **Harvest <10^7 cells grown in suspension into a centrifuge tube. Adherent cells can be lysed directly in cell-culture vessels or trypsinized from culture flasks and collected into a centrifuge tube.**
   b. **Centrifuge at 13,000xg for 10 sec (or 300xg for 5min) to pellet cells. Completely aspirate the supernatant.**
   Note: Incomplete removal of the supernatant will decrease the yield and purity.
   c. **Loosen the cell pellet thoroughly by flicking the tube. Add 350 µl (<$5x10^6$ cells) or 600 µl ($5x10^6$-$1x10^7$ cells) of Buffer RLT Plus, pipet or vortex to mix.**
   d. **Homogenization: (cells <$1x10^5$ can be homogenized by vortexing for 1 min.) Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Homogenization shears genomic DNA, reduces the viscosity of the lysates, and increases the yields.**
   e. **Apply the homogenized lysate to a DNA clearance column placed in a 2 ml collection tube (supplied).**
   f. **Immediately proceed to Step 3.**

2. **Animal tissues (for example, mouse liver and brain)**
   a. **Mince fresh tissues into small pieces, add 350 µl (<20 mg tissue) or 600 µl (20-30mg tissue) of Buffer RLT Plus. Homogenize with electronic tissue homogenizer for 20-40 s. Or**
   b. **Immediately place the tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Transfer adequate amount (20mg/30mg) of tissue powder in to a 1.5 ml microcentrifuge tube containing 350 µl/600 µl of Buffer RLT Plus, vortex for 20 s. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe or homogenize with an electronic tissue homogenizer. This step shears genomic DNA, reduces the viscosity of the lysates, and increases the yields.**

c. Centrifuge the homogenized lysate at 13,000 rpm for 3 min. Transfer the supernatant into a DNA clearance column carefully in a provided 2 ml collection tube.
d. Immediately proceed to Step 3.

3. Centrifuge at 13,000 rpm for 60s, and save the flow-through (RNA is in the flow-through). Ensure all the samples flow through the column. Increase centrifuge speed and time, if necessary.

4. Add equal volume of 70% ethanol (usually 350 µl/600 µl, adjust the ethanol volume accordingly if some lysate is lost during the above procedure) and pipet to mix immediately. Precipitation may be formed after the addition of ethanol, but this does not affect the procedure. Do not centrifuge.

5. Transfer up to 700 µl mixture into a RNA binding column (RA column) placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 30 s, and discard the flow-through. Repeat this step if the sample volume exceeds 700 µl.

6. Add 700 µl Buffer RW1, and incubate at room temperature for 1 min. Centrifuge at 12,000 rpm for 30s. Discard the flow-through.

7. Add 500 µl Buffer RW, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat Step 7 with another 500 µl Buffer RW.

8. Place the RA column back into the same collection tube. Centrifuge the empty RA column at 13,000 rpm for 2 min to completely remove ethanol from the column.

9. Place the RA column in a RNase free microcentrifuge tube. Add 30-50 µl of RNase free water (pre-warm the water to 70-90°C will increase the RNA yield) to the center of the column membrane. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 1 min to elute the RNA.

10. If the expected RNA yield is >30 µg, repeat step 9 with another 30–50 µl of RNase-free water, or using the eluate from step 9 (if high RNA concentration is required). Reuse the centrifuge tube from step 9.
    The RNA yield will be 15–30% higher if using a second volume of RNase-free water than that obtained using the eluate from step 9, but the final RNA concentration will be lower.

Ordering Information

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Purchase Order Number (If any)

Billing address

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Description of the order