CYRN68-CHANGYU EASYspin Plus RNA Micro Kit

Catalog number	Package
CYRN6801	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Poly Carrier is shipped at room temperature, can be stored at 4 °C for one month and should be stored long-term at -20 °C. Keep all solutions tightly capped after use.

Description

This kit is designed for ultra-micro RNA extraction from very small amounts of animal cells, microdissected tissues and easily lysed animal tissues. The typical input is 1 to 5×10^5 cells or less than 5 mg of tissue. The DNA-clear/RNA-bind universal column and dedicated formulation remove residual gDNA without DNase digestion, and the resulting RNA can be used directly in reverse transcription and real-time PCR or in high-throughput sequencing library preparation.

Kit components

Component	Storage	50 preps (CYRN6801)
Lysis Buffer RLT Plus	15-25 °C	25 ml
Deproteinization Buffer RW1	15-25 °C	35 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
70% ethanol	15-25 °C	9 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
Poly Carrier	-20 °C	200 µl
Ultra-micro DNA-clear/RNA-bind universal columns and collection tubes	15-25 °C	100 sets
Micro pestles	15-25 °C	3

Features

A gasket-free column design ensures no residual liquid or contamination after centrifugation, giving highly pure recovered RNA.

The ultra-micro column has a much smaller membrane area, minimizing RNA loss on the membrane, and allows elution in as little as 6 µl, giving high RNA concentration.

The column uses a hydrophobic, nucleic-acid-non-binding support under the silica membrane that withstands centrifugation up to 20,000 rpm without membrane detachment, allowing impurities, salts and ethanol to be removed as completely as possible.

The DNA-clear/RNA-bind universal column removes gDNA in a 1-minute filtration step, so the RNA requires no DNase digestion and can be used directly in reverse transcription, real-time PCR and high-throughput sequencing library preparation.

No toxic phenol or chloroform and no ethanol precipitation step are required.

Notes before starting

Note: All centrifugation steps are carried out at room temperature.

Note: Do not exceed the capacity of the DNA-clear/RNA-bind universal column; otherwise DNA residue or reduced yield will result. Use no more than 5×10^5 cells or 5 mg of tissue.

Note: The kit uses an optimized ultra-micro column and can in principle extract RNA from a single cell.

Note: Lysis Buffer RLT Plus and Deproteinization Buffer RW1 contain irritants. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: The EASYspin Plus buffer system and universal column remove most DNA, so the RNA generally needs no DNase digestion. If the downstream application is very sensitive to trace DNA, treat the RNA with RNase-free DNase I (CYRN45) or perform on-column DNase digestion during extraction (CYRN34).

❖ Procedure

Read the notes before starting. Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW and to the 70% ethanol bottle. If processing fewer than 5000 cells or less than 10 μg of tissue, add 4 μl of Poly Carrier to the lysis buffer before homogenization.

1. Cultured cells

A1. Adherent cells: no trypsinization is needed. Remove the medium completely, add 350 μl of Lysis Buffer RLT Plus and pipette repeatedly to lyse the cells. Load the entire lysate onto a DNA-clear/RNA-bind universal column placed in a collection tube and proceed directly to step 3. For vessels that cannot be lysed directly, scrape the cells or trypsinize and collect them into a 1.5 ml centrifuge tube.

A2. Suspension cells: collect fewer than 5×10^5 cells into a 1.5 ml centrifuge tube.

B. Centrifuge at 13,000 rpm for 10 seconds (or at 300 × g for 5 minutes) to pellet the cells and remove the supernatant completely; incomplete removal dilutes the lysis buffer and reduces yield and purity.

C. Flick the bottom of the tube to loosen the pellet, add 350 μl of Lysis Buffer RLT Plus and pipette repeatedly until no cell clumps remain.

D. Load the entire lysate onto a DNA-clear/RNA-bind universal column placed in a collection tube and leave at room temperature for 1-2 minutes.

E. Proceed immediately to step 3.

2. Animal tissue (for example mouse liver or brain)

A1. Electric homogenization: add 350 μl of Lysis Buffer RLT Plus to less than 5 mg of tissue and homogenize thoroughly for 20-40 seconds.

A2. Pestle homogenization: add 100 μl of Lysis Buffer RLT Plus to a 1.5 ml centrifuge tube, add less than 5 mg of tissue and homogenize immediately with a micro pestle. Make up the lysis buffer to 350 μl.

A3. Liquid nitrogen grinding: grind tissue in liquid nitrogen to a fine powder, transfer an appropriate amount (less than 5 mg) to a 1.5 ml centrifuge tube containing 350 μl of Lysis Buffer RLT Plus and vortex for 20 seconds. For difficult samples, pipette repeatedly or use a pestle to aid homogenization.

B. Load the entire homogenate onto a DNA-clear/RNA-bind universal column placed in a collection tube and leave at room temperature for 1-2 minutes.

C. Proceed immediately to step 3.

3. Centrifuge at 13,000 rpm for 1 minute and keep the filtrate, which contains the RNA.

4. Add an equal volume (about 350 μl) of 70% ethanol (check that ethanol has been added) to the filtrate. A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge.

5. Immediately load the mixture onto a new DNA-clear/RNA-bind universal column placed in a collection tube and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through.

6. Add 700 μl of Deproteinization Buffer RW1, leave at room temperature for 30 seconds, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 μl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 15 seconds and discard the flow-through. Repeat once with another 500 μl of Wash Buffer RW.

8. Return the universal column to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

9. Remove the universal column and place it in a clean 1.5 ml centrifuge tube. Apply 10-20 μl of RNase-free water to the centre of the membrane (prewarming to 80-100 °C improves yield), leave at room temperature for 1 minute and centrifuge at 13,000 rpm for 1 minute to obtain the RNA solution. Reducing the elution volume increases RNA concentration, but the minimum elution volume should not be less than 6 μl.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.