CYRN07-CHANGYU EASYspin Tissue & Cell RNA Kit

Catalog number	Package
CYRN0701	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Do not store at 4 °C or -20 °C: low-temperature storage can cause precipitation of the buffers.

Description

This kit isolates total RNA from fresh or frozen animal tissues and cultured cells using a silica spin-column and a dedicated buffer system. No phenol, chloroform or β-mercaptoethanol is required and no ethanol precipitation step is needed. A single cell sample is typically processed in about 8 minutes.

Kit components

Component	Storage	50 preps (CYRN0701)
Lysis Buffer RLT	15-25 °C	30 ml
Wash Buffer RW1	15-25 °C	35 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
RNA spin columns and collection tubes	15-25 °C	50 sets

Before first use add the volume of absolute ethanol indicated on the Wash Buffer RW label and tick the box on the label.

Features

No toxic phenol/chloroform or β-mercaptoethanol, and no ethanol precipitation.

Fast and simple: a single cell sample is usually completed within 8 minutes.

Typical 28S:18S ratio in agarose gels is greater than 2:1.

Repeated column washes give typical A260/A280 ratios of 2.1-2.2.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a standard benchtop centrifuge.

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

Note: Do not exceed the binding capacity of the column. DNA- and RNA-rich samples differ widely: more than 5 mg of thymus or spleen exceeds the column capacity, and more than 3×10^6 COS cells exceed it. When the nucleic acid content of a sample is unknown, start with no more than $3\text{-}4\times10^6$ cells or 10 mg of tissue and adjust afterwards.

Note: Trace genomic DNA cannot be excluded completely by any total-RNA procedure. Where downstream work is sensitive to trace DNA, use intron-spanning primers, primer pairs that give different product sizes on genomic DNA and cDNA, or treat the RNA with RNase-free DNase I; on-column DNase I digestion is also possible before the RW1 wash.

❖ Sample preparation

A. Adherent cells: remove the medium and add 500 µl Lysis Buffer RLT directly to the dish (up to 8×10^6 cells), pipetting to aid lysis. Cells that remain attached after lysis is complete do not indicate incomplete lysis. For vessels that cannot be lysed directly, scrape the cells or trypsinise, collect by centrifugation, remove the supernatant and add 500 µl Buffer RLT to the pellet.

B. Suspension cells: centrifuge at 13,000 rpm for 10 seconds, remove the supernatant and add 500 µl Lysis Buffer RLT (up to 8×10^6 cells). Vortex or pipette until no cell clumps remain.

C. Animal tissue: take 15-25 mg fresh tissue (not more than 30 mg) and add 500 µl Lysis Buffer RLT. Homogenise with a glass or electric homogeniser. When grinding in liquid nitrogen, transfer 15-25 mg of the powder (not more than 30 mg) to a 1.5 ml tube pre-filled with 500 µl Lysis Buffer RLT and vortex or pipette until no clumps remain. If insoluble debris is abundant, centrifuge the lysate at 13,000 rpm for 3 minutes and transfer the supernatant to a new tube.

❖ RNA extraction

1. Add 0.5 volume of absolute ethanol (about 250 µl) to the lysate and mix by vortexing or pipetting. A precipitate or haze after ethanol addition is normal; mix immediately and do not centrifuge.

2. Transfer the mixture to an RNA spin column in a collection tube. Stand for 1 minute, centrifuge at 13,000 rpm for 30 seconds, discard the flow-through and return the column to the tube.

3. Add 700 µl Wash Buffer RW1, stand at room temperature for 30 seconds, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

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

5. Repeat step 4 once.

6. Return the column to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer.

7. Transfer the column to a new 1.5 ml tube. Apply 30-50 µl RNase-free ddH2O to the centre of the membrane, stand for 1 minute, centrifuge at 13,000 rpm for 1 minute. An elution volume below 30 µl reduces recovery. Pre-warming the water to 70-90 °C or reloading the first eluate increases the RNA concentration.

8. Use the RNA directly in downstream applications or store at -85 °C to -65 °C.

Checking RNA integrity and purity

Integrity: run the RNA on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes and view under UV. Animal 28S and 18S rRNA appear at about 2 kb and 1 kb; the larger band should be 1.5-2.0 times as bright as the smaller one. A smear or loss of the rRNA bands indicates degradation.

Purity: A260/A280 of 2.1-2.2 indicates high-quality RNA. The reading depends on the instrument and on the pH of the diluent; a micro-volume spectrophotometer usually needs no dilution. Concentration: RNA (ng/µl) = A260 × dilution factor × 40.

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