CYRN64-CHANGYU Cytoplasmic & Nuclear RNA Purification Kit

Catalog number	Package
CYRN6401	100 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Do not store at 4 °C or -20 °C, as precipitation affects performance. Keep all solutions tightly capped after use.

Applications

For rapid isolation of cytoplasmic and nuclear RNA from fresh cultured cells and tissues.

Kit components

Component	Storage	100 preps (CYRN6401)
Buffer RLN	15-25 °C	20 ml
Buffer RLN (No NP40)	15-25 °C	20 ml
Lysis Buffer RLT Plus	15-25 °C	50 ml
Deproteinization Buffer RW1	15-25 °C	70 ml
Wash Buffer RW	15-25 °C	20 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
RNase-free spin columns RA and collection tubes	15-25 °C	100 sets

Description

A specially formulated lysis buffer selectively lyses the plasma membrane and releases cytoplasmic RNA. Cytoplasmic RNA is recovered from the supernatant after centrifugation, and nuclear RNA is extracted from the remaining nuclear pellet, allowing cytoplasmic and nuclear RNA to be isolated separately.

Features

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

Simple and rapid: a single sample is usually processed within 25 minutes.

The genomic DNA removal column technology and specially formulated buffers effectively remove residual gDNA. Compared with imported kits of the same type, residual gDNA in nuclear RNA is substantially reduced.

Repeated column washes ensure high purity. Typical A260/A280 is 2.1-2.2 with little residual DNA, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: Use fresh cell or tissue samples.

Note: All centrifugation steps can be performed at room temperature at 13,000 rpm in a conventional benchtop centrifuge.

Note: Ethanol and an optional mortar must be supplied by the user.

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 kit provides enough material for 100 cytoplasmic RNA samples or 50 nuclear RNA samples, because each nuclear RNA sample requires two RNA spin columns. To process 50 cytoplasmic and 50 nuclear samples at the same time, an additional 50 RNA spin columns must be ordered separately.

Note: Buffer RLN contains 0.5% NP40, a mild non-ionic detergent that selectively lyses the plasma membrane without lysing the nuclear membrane. If a particular cell type is over- or under-lysed and separation is poor, Buffer RLN (No NP40) is supplied so that a series of RLN buffers with different NP40 concentrations (for example 0.4%, 0.5%, 0.6%, 0.7%, 0.8%) can be prepared to optimize lysis conditions; lysis time can also be adjusted for the best separation.

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW as indicated on the label. Optional: add 1 mM DTT to Buffer RLN just before use. Optional: add 1000 U/ml RNase inhibitor to Buffer RLN just before use.

(A) Sample lysis

1. Cultured cells. A1. Adherent cells: for cells grown in dishes smaller than 3.5 cm in diameter, no trypsinization is needed; remove the medium completely, wash once with 1× PBS, remove the liquid completely and proceed 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 10^7 cells into a 1.5 ml centrifuge tube. B. Centrifuge at 300 × g for 5 minutes and remove the supernatant completely, leaving the cell pellet. Incomplete removal of supernatant dilutes the lysis buffer and reduces yield and purity. C. Flick the bottom of the tube to loosen the pellet and proceed immediately to step 3.

2. Small tissue samples. A. Liquid nitrogen grinding and homogenization: cut 10-15 mg of tissue into a mortar, grind to powder in liquid nitrogen and, before the powder thaws, add 175 µl of Buffer RLN (precooled on ice or at 4 °C) and pipette gently with a wide-bore tip until the tissue is completely lysed. Transfer the lysate to a new centrifuge tube. B. Proceed immediately to step 4.

3. Add 175 µl of Buffer RLN (precooled on ice or at 4 °C) to lyse the cells. For a culture dish, add Buffer RLN, rock to cover all cells, scrape gently with a rubber scraper, transfer to a 1.5 ml centrifuge tube and lyse on ice for 5 minutes. For a collected cell pellet, flick the tube to disperse the pellet, add Buffer RLN and resuspend the cells gently and completely, then lyse on ice for 5 minutes, inverting once or twice.

4. Centrifuge at 4 °C at 300 × g for 2 minutes. Transfer the supernatant (containing cytoplasmic RNA) to a new tube and keep the pellet (containing nuclear RNA). Add 400 µl of Lysis Buffer RLT Plus to the pellet, vortex to mix and keep on ice. Depending on cell type and number, a visible nuclear pellet may sometimes be absent.

(B) Cytoplasmic RNA extraction (all steps at room temperature)

1. Add 600 µl of Lysis Buffer RLT Plus to the supernatant obtained in step 4 of sample lysis and vortex to mix. Add 430 µl of absolute ethanol and mix by pipetting.

2. Load the mixture (no more than 720 µl per load; larger volumes can be loaded in two portions) immediately onto spin column RA placed in a collection tube. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

3. 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.

4. 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.

5. Return column RA 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.

6. Remove column RA and place it in a clean 1.5 ml centrifuge tube. Apply 30-50 µl of RNase-free water to the centre of the membrane, leave at room temperature for 1 minute and centrifuge at 13,000 rpm for 1 minute to obtain the RNA solution. The elution volume should be at least 30 µl; smaller volumes reduce recovery. For a higher RNA concentration, reload the eluate onto the column and repeat the elution.

(C) Nuclear RNA extraction (all steps at room temperature)

1. Pipette the resuspended pellet from step 4 of sample lysis (to which 400 µl of Lysis Buffer RLT Plus has been added) onto a spin column RA placed in a collection tube. Centrifuge immediately at 13,000 rpm for 1 minute and keep the flow-through, which contains the nuclear RNA.

2. Add 0.5 volume of ethanol to the flow-through. A precipitate may form; it does not affect the procedure. Mix immediately by pipetting and do not centrifuge.

3. Load the mixture immediately onto a new column RA placed in a collection tube. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

4. Continue from step 3 of section (B) Cytoplasmic RNA extraction and complete the remaining steps.

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