CYRN41-CHANGYU EASYspin Plus Large-Scale Tissue/Cell RNA Kit

Catalog number	Package
CYRN4101	10 preps

Storage and shipping: 15-25 °C. Stable for 6 months. Solutions should be clear; if a precipitate forms at low temperature, heat in a 37 °C water bath for a few minutes until clear. Do not store at 4 °C or -20 °C. Keep all solutions tightly capped after use.

Applications

For rapid extraction of total RNA from animal cells and easily lysed animal tissues. The dedicated genomic DNA removal column effectively removes residual gDNA, so no DNase digestion is needed and the RNA can be used directly in PCR and real-time PCR.

Kit components

Component	Storage	10 preps (CYRN4101)
Lysis Buffer RLT Plus	15-25 °C	100 ml
Deproteinization Buffer RW1	15-25 °C	120 ml
Wash Buffer RW	15-25 °C	25 ml × 2 (add ethanol before first use)
70% ethanol	15-25 °C	15 ml × 2 (add ethanol before first use)
RNase-free H2O	15-25 °C	10 ml
Genomic DNA removal columns and collection tubes	15-25 °C	10 sets
RNase-free spin columns RA and collection tubes	15-25 °C	10 sets

Description

Based on the phenol- and chloroform-free EASYspin technology, this kit adds a genomic DNA removal column that effectively removes residual gDNA, so the RNA requires no DNase digestion and can be used directly in PCR and real-time PCR. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases. The lysate passes through a genomic DNA removal column that retains genomic DNA while RNA flows through. The filtered RNA is adjusted with ethanol and binds selectively to the silica membrane under high-ionic-strength conditions; rapid wash and centrifugation steps remove metabolites, proteins and other impurities, and purified RNA is eluted in low-salt RNase-free H2O.

Features

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

Fast and simple: a single sample is usually processed within 60 minutes.

The genomic DNA removal column effectively removes residual gDNA, so the RNA requires no DNase digestion and can be used directly in PCR and real-time PCR.

Repeated column washes ensure high purity; typical A260/A280 is 1.9-2.0 with little residual DNA, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: All centrifugation steps can be carried out at room temperature in a centrifuge that accepts 50 ml tubes.

Note: Do not exceed the capacity of genomic DNA column DA or RNA column RA; otherwise DNA residue or reduced yield will result. RNA/DNA content differs greatly between tissues: more than 100 mg of thymus or spleen exceeds column capacity, and more than 6×10^7 COS cells exceed column capacity. When establishing conditions for an unknown sample, start with less material, for example no more than 6×10^7 cells or 200 mg of tissue, and adjust afterwards.

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: Trace genomic DNA cannot be excluded completely by any total-RNA procedure. The EASYspin buffer system and membrane remove most DNA, and trace DNA (generally not visible after ethidium bromide staining under UV) has little effect in most RT-PCR amplifications. For stringent mRNA expression analysis such as real-time PCR: (1) use intron-spanning primers; (2) use primer pairs that give different product sizes on genomic DNA and cDNA; (3) treat the RNA with RNase-free DNase I; the kit can also clean up RNA after DNase I treatment; (4) perform DNase I treatment on column RA before the RW1 wash.

Note: RNA integrity: run on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes. Because 70-80% of cellular RNA is rRNA, prominent rRNA bands should be visible under UV. Animal rRNA is about 5 kb and 2 kb (28S and 18S); the largest rRNA band should be 1.5-2.0 times as bright as the next largest, otherwise the RNA is degraded. Purity: high-quality RNA gives A260/A280 of 1.8-2.1 in 10 mM Tris, pH 7.5; readings in water may be 1.5-1.9. Concentration: (ng/μl) = A260 × dilution factor n × 40.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW and to the 70% ethanol bottle.

1. Cultured cells

a. Collect fewer than 2×10^8 suspension cells into an appropriately sized centrifuge tube. For adherent cells, cells in plates can be lysed directly; cells in flasks should be trypsinized and collected by pipetting.

b. Centrifuge at 10,000-13,000 × g for 20 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 tube to loosen and resuspend the pellet completely. Add 5 ml (fewer than 10^8 cells) or 10 ml (1×10^8-2×10^8 cells) of Lysis Buffer RLT Plus, mix by pipetting and vortex vigorously for 20 seconds.

d. Homogenize: for very few cells (fewer than 2×10^6), vortexing for 1 minute is usually sufficient. Otherwise pass the lysate 10 or more times through a disposable 10 ml syringe with a blunt 0.9 mm needle, or until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 60 seconds) to shear DNA, reduce viscosity, prevent column clogging and increase yield.

e. Load the entire lysate or homogenate onto a DNA removal column placed in a collection tube.

f. Proceed immediately to step 3.

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

a. Electric homogenization: cut fresh tissue quickly into small pieces, add 5 ml (less than 250 mg tissue) or 10 ml (400-500 mg tissue) of Lysis Buffer RLT Plus and homogenize thoroughly for 1 minute.

b. Liquid nitrogen grinding: grind tissue in liquid nitrogen to a fine powder, transfer 250 mg or 500 mg of powder to a 50 ml centrifuge tube containing 5 ml or 10 ml of RLT Plus and vortex vigorously for 20

seconds. Pass the lysate 10 times through a disposable 10 ml syringe with a blunt 0.9 mm needle, or until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 60 seconds).

c. Centrifuge the homogenate at 10,000-13,000 × g for 5 minutes to pellet difficult debris or insoluble material, then load all of the supernatant onto a DNA removal column placed in a collection tube.

d. Proceed immediately to step 3.

3. Centrifuge immediately at 10,000-13,000 × g for 5 minutes and keep the filtrate, which contains the RNA. Make sure all liquid has passed through the membrane; if necessary, increase the centrifugal force and time.

4. Estimate the filtrate volume accurately with a micropipette (usually 5 ml or 10 ml, minus volume lost during filtration) and add an equal volume of 70% ethanol (check that ethanol has been added). A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge.

5. Load the mixture onto spin column RA placed in a collection tube and centrifuge at 10,000-13,000 × g for 3 minutes (make sure all liquid has passed through and nothing remains on the membrane; otherwise increase speed and time). Discard the flow-through.

6. Add 10 ml of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 12,000 × g for 3 minutes and discard the flow-through.

7. Add 10 ml of Wash Buffer RW (check that ethanol has been added), centrifuge at 10,000-13,000 × g for 1-2 minutes and discard the flow-through. Repeat once with another 10 ml of Wash Buffer RW.

8. Return column RA to the empty collection tube and centrifuge at 13,000 × g for 5 minutes to dry the membrane. Use a pipette tip to remove any residual ethanol between the inner ring and the column wall, then air-dry at room temperature or in an oven for a few minutes.

9. Remove column RA and place it in an RNase-free centrifuge tube. Apply 500 μl-1 ml of RNase-free H2O to the centre of the membrane, leave at room temperature for 3 minutes and centrifuge at 12,000 × g for 2 minutes.

10. If the expected RNA yield is greater than 0.6 mg, add 300-500 μl of RNase-free H2O and repeat step 9, then combine the two eluates; alternatively, reload the first eluate onto the column if a higher concentration is needed. Two separate elutions give 15-30% more total RNA but a lower concentration than a single elution with the combined first eluate; choose according to your needs.

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