CYRN29-CHANGYU Allprep Tissue/Cell RNA/DNA Kit

Catalog number	Package
CYRN2901	20 preps
CYRN2902	50 preps

Storage and shipping: 15-25 °C. Stable for 12 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 simultaneous extraction and separation of genomic DNA and total RNA from the same animal cell or tissue sample. No DNase digestion is required, and the RNA can be used directly in reverse transcription PCR and real-time PCR.

Kit components

Component	20 preps (CYRN2901)	50 preps (CYRN2902)
Lysis Buffer RLT Plus	20 ml	50 ml
Deproteinization Buffer RW1	15 ml	40 ml
Wash Buffer RW	5 ml	10 ml (add ethanol before first use)
RNase-free H2O	10 ml	10 ml
70% ethanol	4 ml	9 ml (add ethanol before first use)
Inhibitor Removal Solution IR	10 ml	25 ml
Wash Buffer WB	15 ml	15 ml (add ethanol before first use)
Elution Buffer EB	10 ml	10 ml
Genomic DNA spin columns DA and collection tubes	20 sets	50 sets
RNA spin columns RA and collection tubes	20 sets	50 sets

Description

This kit simultaneously extracts and separates genomic DNA and total RNA from the same animal cell or tissue sample. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases and DNases. The lysate passes through a genomic DNA spin column: genomic DNA binds while RNA flows through. Bound genomic DNA is washed, centrifuged and eluted to give pure genomic DNA. The filtered RNA is adjusted with ethanol and binds selectively to the RNA spin column under high-ionic-strength conditions; rapid wash and centrifugation steps give pure RNA. Based on phenol- and chloroform-free extraction and a dedicated separation technology, the RNA and genomic DNA are highly pure and do not interfere with each other. The RNA requires no DNase digestion and can be used directly in reverse transcription PCR and real-time PCR; genomic DNA is suitable for Southern blot, restriction digestion, PCR and other applications.

Features

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

Fast and simple: separation of RNA and genomic DNA from a single sample is usually completed within 40 minutes.

The dedicated column and formulation effectively remove residual genomic DNA; the RNA generally requires no DNase digestion and can be used directly in reverse transcription PCR and real-time PCR.

Repeated column washes ensure high purity of RNA and genomic DNA for downstream applications.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C.

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 5 mg of thymus or spleen exceeds column capacity, and more than 3×10^6 COS cells exceed column capacity. When establishing conditions for an unknown sample, start with less material, for example no more than $3\text{-}4\times10^6$ cells or 10 mg of tissue, and adjust afterwards.

Note: Lysis Buffer RLT Plus, Inhibitor Removal Solution IR 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: To prevent RNase contamination: change gloves frequently, use RNase-free plasticware and tips, bake glassware at 150 °C for 4 hours and soak plasticware in 0.5 M NaOH for 10 minutes followed by thorough rinsing and sterilization, and prepare solutions with RNase-free water (DEPC 0.1% v/v, 37 °C overnight, autoclave).

Note: Trace genomic DNA cannot be excluded completely by any total-RNA procedure, and even DNase digestion is not 100% effective. The Allprep buffer system and genomic DNA separation technology remove most DNA, so the RNA generally requires no DNase digestion and can be used directly in reverse transcription PCR and real-time PCR. For samples with very high DNA content or for stringent mRNA expression analysis by real-time PCR: use intron-spanning primers or primer pairs that give different product sizes on genomic DNA and cDNA; treat the RNA with RNase-free DNase I; the kit can also clean up RNA after DNase I treatment; on-column DNase I digestion on column RA before the RW1 wash is also possible.

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

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

1. Cultured cells

a. Collect fewer than 10^7 suspension cells into a 1.5 ml 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 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 tube to loosen and resuspend the pellet completely. Add 350 µl (fewer than 5×10^6 cells) or 600 µl (5×10^6-1×10^7 cells) of Lysis Buffer RLT Plus, mix by pipetting and vortex vigorously for 20 seconds.

d. Homogenize: for very few cells (fewer than 10^5), vortexing for 1 minute is usually sufficient. Otherwise pass the lysate 10 or more times through a disposable 1 ml syringe with a blunt 0.9 mm needle, or until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds).

e. Load the entire lysate or homogenate onto a DNA spin 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 350 µl (less than 20 mg tissue) or 600 µl (20-30 mg tissue) of Lysis Buffer RLT Plus and homogenize thoroughly for 20-40 seconds.

b. Liquid nitrogen grinding and homogenization: grind tissue in liquid nitrogen to a fine powder, transfer 20 mg or 30 mg of powder to a 1.5 ml centrifuge tube containing 350 µl or 600 µl of RLT Plus and vortex vigorously for 20 seconds. Pass the lysate 10 times through a disposable 1 ml syringe with a blunt 0.9 mm needle, or until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds).

c. Centrifuge the homogenate at 13,000 rpm for 3 minutes to pellet difficult debris or insoluble material, then load all of the supernatant onto a DNA spin column placed in a collection tube.

d. Proceed immediately to step 3.

3. Centrifuge at 14,000 rpm for 60 seconds and keep the flow-through, which contains RNA. The DNA spin column (with genomic DNA bound to the membrane) can be stored briefly at 4 °C for later use. Make sure all liquid has passed through the membrane; if necessary, increase the centrifugal force and time.

RNA extraction (steps 4-10)

4. Estimate the flow-through volume accurately with a micropipette (usually 350 µl or 600 µl, 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. Immediately load the mixture (no more than 700 µl per load; larger volumes can be loaded in two portions) onto RNA spin column RA placed in a collection tube and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through. Keep the collection tube that held the DNA spin column, return the DNA spin column to it and keep at 4 °C for genomic DNA extraction starting at step 11.

6. Add 700 µl of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 12,000 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 12,000 rpm for 30 seconds and discard the flow-through. Repeat once with another 500 µl of Wash Buffer RW.

8. 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.

9. Remove column RA and place it in an RNase-free centrifuge tube. Apply 30-50 µl of RNase-free water to the centre of the membrane (prewarming to 70-90 °C improves yield), leave at room temperature for 1 minute and centrifuge at 12,000 rpm for 1 minute.

10. If the expected RNA yield is greater than 30 µg, add 30-50 µl of RNase-free water 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.

Genomic DNA extraction (steps 11-16)

11. Add 500 µl of Inhibitor Removal Solution IR to the DNA spin column from step 3 and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.

12. Add 700 µl of Wash Buffer WB (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

13. Add 500 µl of Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

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

15. Remove the DNA spin column and place it in a clean centrifuge tube. Apply 100 µl of Elution Buffer EB to the centre of the membrane (prewarming EB to 65-70 °C improves elution), leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave for 2 minutes and centrifuge again at 12,000 rpm for 1 minute. A larger elution volume increases elution efficiency; a smaller volume gives a higher DNA concentration, but the minimum volume should not be less than 50 µl.

16. DNA can be stored at 2-8 °C or, for long-term storage, at -20 °C.

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