CYRN36-CHANGYU Allprep Tissue/Cell RNA/DNA/Protein Kit

Catalog number	Package
CYRN3601	20 preps
CYRN3602	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, total RNA and protein 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 (CYRN3601)	50 preps (CYRN3602)
Lysis Buffer RLT	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)
Buffer APP	25 ml	60 ml
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, total RNA and protein 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 and protein flow 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. Protein is recovered from the filtrate by selective precipitation. 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, genomic DNA and protein from a single sample is usually completed within 1 hour.

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, 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: For plant samples, especially difficult samples rich in polysaccharides and polyphenols, consult technical support before using this kit; additional reagents may be required.

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.

❖ 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, 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 5-10 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) to shear DNA, reduce viscosity, prevent column clogging and increase yield.

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

f. Proceed 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 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 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 to step 3.

3. Centrifuge at 13,000 rpm for 30 seconds and keep the flow-through, which contains RNA and protein. 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. Keep the flow-through for protein extraction starting at step 17. 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.

Protein extraction (steps 17-21)

17. Add an equal volume of Buffer APP to the flow-through from step 5, vortex to mix and leave at room temperature for 15 minutes to precipitate protein.

18. Centrifuge at 13,000 rpm for 5-10 minutes and carefully discard the supernatant. Add 0.5 ml of 70% ethanol, invert, centrifuge for 1 minute, carefully discard the supernatant and keep the protein pellet; remove residual liquid as completely as possible with a pipette.

19. Air-dry the pellet at room temperature for 5-10 minutes; ensure that ethanol has evaporated completely so that it does not affect downstream experiments.

20. Dissolve the protein pellet in 30-150 µl of 1× protein loading buffer (omit bromophenol blue if protein quantification is required) or another buffer required for downstream experiments. Because of the strong denaturing effect of Lysis Buffer RLT and the different isoelectric points of proteins, dissolution may be difficult; pipette to help, adjust the pH, or briefly centrifuge and take the supernatant. If necessary, use 5% SDS or 8 M urea to aid dissolution. For BCA protein quantification, 8 M urea may need to be diluted to 3 M.

21. Heat at 95 °C for 5 minutes to dissolve and denature the protein, cool to room temperature, centrifuge at maximum speed for 1 minute and use the supernatant for SDS-PAGE or Western blot.

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