CYRN48-CHANGYU Allprep Tissue/Cell miRNA/RNA/DNA Kit

Catalog number	Package
CYRN4801	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 (including miRNA) from the same animal cell or tissue sample. If additional miRNA spin columns and solutions are purchased, total RNA and miRNA from the same sample can be separated and miRNA enriched. No DNase digestion is required, and the RNA can be used directly in reverse transcription PCR and real-time PCR.

Kit components

Component	Storage	50 preps (CYRN4801)
Lysis Buffer RLT Plus	15-25 °C	50 ml
Wash Solution 1	15-25 °C	12 ml (add 28 ml absolute ethanol before first use)
Wash Solution 2/3	15-25 °C	10 ml (add 42 ml absolute ethanol before first use)
RNase-free H2O	15-25 °C	10 ml
Inhibitor Removal Solution IR	15-25 °C	25 ml
Wash Buffer WB	15-25 °C	15 ml (add ethanol before first use)
Elution Buffer EB	15-25 °C	10 ml
Genomic DNA spin columns DA and collection tubes	15-25 °C	50 sets
RNA spin columns RA and collection tubes	15-25 °C	50 sets

Description

This kit simultaneously extracts and separates genomic DNA and total RNA (including miRNA) from the same animal cell or tissue sample. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases and DNases. The lysate is passed through a genomic DNA spin column: genomic DNA binds to the column while miRNA/RNA flows through. Bound genomic DNA is washed, centrifuged and eluted to give pure genomic DNA. The filtered miRNA/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 miRNA/RNA. Based on phenol- and chloroform-free nucleic acid extraction and a dedicated separation technology, the miRNA/RNA and genomic DNA are highly pure and do not interfere with each other. The miRNA/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 miRNA/RNA/genomic DNA from a single sample is usually completed within 40 minutes.

The dedicated column and formulation effectively remove residual genomic DNA; the miRNA/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 miRNA/RNA/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, Wash Solution 1 and Wash Solution 2/3 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: If additional miRNA spin columns and solutions are purchased, total RNA and miRNA from the same sample can be separated and miRNA enriched; please inquire.

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.

❖ Procedure

Read the notes before starting. Before first use, add the indicated amount of absolute ethanol to Wash Solution 1, Wash Solution 2/3 and Wash Buffer WB.

1. Cultured cells

a. Collect fewer than 10^7 suspension cells into a 1.5 ml centrifuge tube. For adherent cells, cells grown in plates can be lysed directly; cells grown 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 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 Plus and homogenize thoroughly for 20-40 seconds.

b. Liquid nitrogen grinding and homogenization: grind tissue in liquid nitrogen to a fine powder, transfer an appropriate amount (20 mg or 30 mg) to a 1.5 ml centrifuge tube containing 350 µl or 600 µl of Lysis Buffer 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 to step 3.

3. Centrifuge at 13,000 rpm for 30 seconds and keep the flow-through, which contains miRNA/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 (about 350 µl or 600 µl, minus volume lost during filtration) and add 1.25 volumes of absolute ethanol. 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 (the empty tube left after transferring the mixture to the RNA 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 Wash Solution 1 (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 µl of Wash Solution 2/3 (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 Solution 2/3.

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, leave at room temperature for 1 minute and centrifuge at 12,000 rpm for 1 minute.

10. The RNA can be used immediately in downstream reactions or stored at low temperature as soon as possible.

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, since residual ethanol can inhibit downstream reactions.

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, as smaller volumes reduce elution efficiency and yield.

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.