CYRN05-CHANGYU RNAmis miRNA Rapid Extraction Kit

Catalog number	Package
CYRN0501	50 preps

Storage: Lysis/Binding Buffer at 4 °C protected from light; other components at 15-25 °C. Stable for 6 months when stored as directed. Wash Solution 1 and Wash Solution 2/3 remain usable for one month at room temperature after ethanol is added, or longer at 4 °C (equilibrate to room temperature before use). Shipping at ambient temperature.

Description

The kit rapidly isolates miRNA and other small RNAs of about 15-30 nucleotides, together with total RNA, from cells and tissues. Conventional silica membranes bind small RNAs poorly, and phenol/guanidine extraction or ethanol precipitation does not recover them efficiently. In this kit a lysis buffer containing β-mercaptoethanol lyses cells and inactivates cellular RNases, an organic extraction removes protein and DNA, and RNA including small RNA binds to a silica membrane in the spin column. Fast wash and centrifugation steps remove metabolites and protein, and purified RNA is eluted in a low-salt buffer.

Kit components

Component	Storage	50 preps (CYRN0501)
Lysis/Binding Buffer	4 °C, dark	50 ml
70% Ethanol	15-25 °C	9 ml
Wash Solution 1 (add 28 ml absolute ethanol before first use)	15-25 °C	12 ml
Wash Solution 2/3 (add 42 ml absolute ethanol before first use)	15-25 °C	10 ml
RNase-free H2O	15-25 °C	10 ml
RNA spin columns RA and collection tubes	15-25 °C	50 sets
microRNA spin columns MA and collection tubes	15-25 °C	50 sets

Features

The silica membrane gives low column-to-column variation and reproducible recovery.

No ethanol precipitation step, which would lose small RNAs.

A single sample is usually processed within 30 minutes.

Repeated washes give typical A260/A280 ratios of 1.9-2.0 with essentially no DNA carry-over; suitable for RNAi, RT-PCR, Northern blotting and related applications.

Notes before starting

Note: Add the indicated volume of ethanol to the 70% ethanol, Wash Solution 1 and Wash Solution 2/3 bottles before first use and tick the box on each label.

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a standard benchtop centrifuge.

Note: Ethanol, chloroform, disposable syringes and a mortar are required but not supplied.

Note: Lysis/Binding Buffer and Wash Solution 1 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 plastics and tips, and bake glassware at 150 °C for 4 hours or soak plasticware in 0.5 M NaOH for 10 minutes followed by thorough rinsing and sterilisation. Prepare solutions with DEPC-treated water (0.1% v/v DEPC overnight at 37 °C, then autoclave).

❖ Procedure (total RNA including microRNA)

Note: Add the indicated ethanol to the 70% ethanol, Wash Solution 1 and Wash Solution 2/3 bottles before first use.

1. Cultured cells: collect fewer than 10^7 suspension cells in a 1.5 ml tube and centrifuge at 13,000 rpm for 10 seconds (or 300×g for 5 minutes); remove the supernatant completely. For adherent cells, remove the medium as completely as possible and add 1 ml Lysis/Binding Buffer directly to the dish or flask, swirl gently and pipette to lyse, then continue at step 3.

2. Animal tissue: mince fresh tissue and add 1 ml Lysis/Binding Buffer per 50-100 mg, then homogenise thoroughly. Alternatively grind in liquid nitrogen and transfer 50-100 mg of powder to a 1.5 ml tube containing 1 ml Lysis/Binding Buffer and vortex vigorously. If the lysate is very viscous or contains visible particles, pass it 10 times through a disposable 5 ml syringe with a 0.9 mm needle (or homogenise electrically for 30 seconds).

3. Stand at room temperature for 5 minutes to dissociate nucleic acid-protein complexes.

4. Add 200 µl chloroform and vortex vigorously for 15 seconds.

5. Stand at room temperature for 2-3 minutes and centrifuge at 13,000 rpm for 10 minutes.

6. Carefully transfer the upper phase (about 600 µl) to a new tube and add 1.5 volumes of room-temperature absolute ethanol (usually about 900 µl); vortex. A precipitate may form; mix immediately and do not centrifuge.

7. Load the mixture (not more than 700 µl at a time) onto an RNA spin column RA in a collection tube and centrifuge at 12,000 rpm for 30-60 seconds; discard the flow-through.

8. Add 700 µl Wash Solution 1 (check that ethanol has been added) and centrifuge at 12,000 rpm for 30 seconds; discard the flow-through.

9. Add 500 µl Wash Solution 2/3 (check that ethanol has been added) and centrifuge at 12,000 rpm for 30 seconds; discard the flow-through. Repeat once.

10. Return the column to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash solution.

11. Transfer the column to an RNase-free tube and apply 30-50 µl RNase-free water to the centre of the membrane (pre-warmed to 100 °C for better recovery). Stand for 1 minute and centrifuge at 12,000 rpm for 1 minute.

12. If more than 30 µg of RNA is expected, repeat step 11 and combine the eluates (higher yield, lower concentration), or reload the first eluate onto the column (higher concentration). A second elution increases yield by 15-30%.

Appendix: microRNA enrichment

This procedure recovers microRNA only, without the other total-RNA components larger than 200 nt. Follow steps 1-5 above to obtain the upper phase. Estimate its volume (about 600 µl), add an equal volume of room-temperature 70% ethanol and mix; do not centrifuge. Load onto an RNA spin column RA, centrifuge at 12,000 rpm for 30-60 seconds and keep the flow-through, which contains the microRNA; the

column retains total RNA without microRNA and can be washed and eluted by steps 8-11 if required. Estimate the flow-through volume, add 0.65 volume of room-temperature absolute ethanol and mix. Load onto a new microRNA spin column MA and centrifuge at 12,000 rpm for 30 seconds; discard the flow-through. Wash and elute as in steps 8-11 to obtain enriched microRNA.

Note: For Northern blotting or expression arrays, total RNA including microRNA can be used. Enriched microRNA reduces the background from mRNA and rRNA and may help when non-specific amplification is a problem.

Checking RNA integrity and purity

Integrity: run the RNA on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes and view under UV. Animal rRNA appears at about 5 kb (28S) and 2 kb (18S); the larger band should be 1.5-2.0 times as bright as the smaller one. A smear or loss of the bands indicates degradation. Purity: A260/A280 of 1.8-2.1 in 10 mM Tris pH 7.5 indicates high-quality RNA. The ratio depends on the pH of the solution: the same RNA may read 1.5-1.9 in water without being impure. Concentration: RNA (ng/µl) = A260 × dilution factor × 40.

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