CYRN59-CHANGYU miEASYspin Universal microRNA Kit (Chloroform-free)

Catalog number	Package
CYRN5901	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. Wash Solution 2/3 may form crystals a few days after adding ethanol; avoid the crystals and use the supernatant. Keep all solutions tightly capped after use.

Applications

For extraction of microRNA or total RNA including microRNA from tissues, cells, ordinary plants and exosomes.

Kit components

Component	Storage	50 preps (CYRN5901)
Lysis Buffer MRL	15-25 °C	25 ml
Wash Solution 1	15-25 °C	10 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
Genomic DNA removal columns and collection tubes	15-25 °C	50 sets
RNase-free spin columns RA and collection tubes	15-25 °C	50 sets

Description

Conventional microRNA kits use guanidinium thiocyanate/phenol/chloroform (TRIzol) extraction followed by binding of small RNAs to a silica membrane in high ethanol. Phenol and chloroform are increasingly restricted because of toxicity, carcinogenicity and environmental concerns, and the TRIzol route is not effective for some samples. This kit uses a phenol- and chloroform-free route. A dedicated universal lysis buffer rapidly lyses cells and inactivates cellular RNases; the lysate passes through a genomic DNA removal column that retains genomic DNA while RNA, including microRNA, flows through. The filtered RNA is adjusted with ethanol and binds selectively to the silica membrane under high-ionic-strength conditions; special wash buffers remove metabolites, proteins and other impurities, and purified RNA, including microRNA, is eluted in low-salt RNase-free H2O.

Features

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

Fast and simple, with far fewer steps and less time than conventional microRNA kits.

Can yield total RNA including microRNA, or separately purify enriched microRNA and total RNA (larger than 200 nt) using the enrichment procedure.

Repeated column washes ensure high purity; typical A260/A280 is 2.0-2.2, suitable for RT-PCR, Northern blot and other applications.

Notes before starting

Note: All centrifugation steps can be carried out at room temperature at 12,000 rpm in a conventional benchtop centrifuge.

Note: Ethanol and an optional mortar must be supplied by the user.

Note: Do not exceed the capacity of genomic DNA removal column DA or RNA column RA; otherwise DNA residue or reduced yield will result. When establishing conditions for an unknown sample, start with less material and adjust afterwards.

Note: Lysis Buffer MRL 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: Trace genomic DNA cannot be excluded completely by any total-RNA procedure, and even DNase digestion is not 100% effective. This kit uses a dedicated buffer system and genomic DNA removal column that remove most DNA. If trace genomic DNA must be removed: (1) digest with conventional DNase, heat-inactivate and use directly; (2) digest with conventional DNase and purify with the RNAclean Plus Enhanced RNA Cleanup Kit including small RNA (CYRN62); (3) perform on-column DNase I digestion on column RA using the CYRN34 DNase I on-column digestion kit (replace Deproteinization Buffer RW1 with Wash Solution 1).

❖ Procedure

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

(1) Sample preparation

1. Cultured cells. a. Suspension cells: collect by centrifugation at 12,000 rpm for 15 seconds, remove the supernatant completely and add 500 µl of Lysis Buffer MRL (fewer than 8×10^6 cells); vortex or pipette to lyse. b. Adherent cells: no trypsinization is needed; remove the medium completely, add 500 µl of MRL (fewer than 8×10^6 cells) and pipette repeatedly. For vessels that cannot be lysed directly, scrape or trypsinize the cells, collect into a 1.5 ml tube, centrifuge, remove the supernatant completely and add 500 µl of MRL.

2. Animal tissue. a. Homogenization: add 500 µl of MRL to 10-25 mg of fresh tissue and homogenize with a glass or electric homogenizer until no obvious tissue pieces remain. b. Liquid nitrogen grinding: grind tissue in liquid nitrogen to a fine powder, transfer 10-25 mg of powder to a 1.5 ml tube containing 500 µl of MRL and vortex until no visible powder clumps remain.

3. Ordinary plants. a. Place 500 µl of MRL in a 1.5 ml tube. b. Grind plant tissue in liquid nitrogen to a fine powder, transfer 50-100 mg of powder to the MRL tube and shake vigorously for 20 seconds. c. Pipette or vortex until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds). d. Centrifuge the lysate at 12,000 rpm for 5-10 minutes to pellet unlysed debris.

(2) Total RNA (including microRNA) extraction

1. Load the entire lysate or the supernatant onto a genomic DNA removal column placed in a collection tube and centrifuge immediately at 12,000 rpm for 1 minute. Collect the filtrate, which contains RNA/microRNA.

2. Estimate the filtrate volume accurately with a micropipette (about 480 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.

3. Immediately load the mixture (no more than 700 µl per load; larger volumes can be loaded in two portions) onto spin column RA placed in a collection tube and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through. Make sure all liquid has passed through the membrane; if necessary, increase

the centrifugal force and time.

4. 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.

5. 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.

6. Return column RA to the empty collection tube and centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer.

7. Remove column RA and place it in a new 1.5 ml 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. Reloading the eluate onto the column for a second elution increases yield by about 10-15%.

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

❖ Appendix 1: Relative microRNA enrichment (separate extraction of microRNA and microRNA-depleted total RNA)

Add ethanol to Wash Solution 1 and Wash Solution 2/3 before first use.

1. Prepare the lysate or supernatant according to sample preparation.

2. Add half a volume of absolute ethanol (0.5 volume) to the lysate or supernatant. A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge.

3. Load the mixture (no more than 720 µl per load) onto a genomic DNA removal column placed in a collection tube and centrifuge at 12,000 rpm for 2 minutes. Keep the filtrate, which contains microRNA. The column retains microRNA-depleted total RNA; if desired, wash and elute it according to standard steps 4-8 to recover microRNA-depleted total RNA.

4. Estimate the filtrate volume accurately and add an equal volume of absolute ethanol (at room temperature). Vortex or pipette thoroughly to mix; do not centrifuge.

5. Immediately load the mixture (no more than 700 µl per load) onto spin column RA placed in a collection tube and centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.

6. Wash and elute according to standard steps 4-8 to obtain enriched microRNA.

Note: Different experiments may require different approaches. For example, Northern blot or expression array analysis may use total RNA that includes microRNA. Enriched microRNA lacks large mRNA and rRNA species and may reduce amplification background in some downstream assays; when background or non-specific amplification is high, enriched microRNA can be tried. In general, use total RNA including microRNA first, and use enrichment only when total RNA performs poorly or when the experimental design requires separation of microRNA and mRNA.

❖ Appendix 2: On-column DNase digestion (see the CYRN34 DNase I on-column digestion kit for details)

1. Follow the standard procedure up to and including step 3.

2. Mix 45 µl of DNase I buffer and 5 µl of RNase-free DNase I gently to prepare the working solution (scale up proportionally for multiple columns).

3. Add 350 µl of Wash Solution 1 to column RA, centrifuge at 12,000 rpm for 30 seconds, discard the flow-through and return the column to the collection tube.

4. Apply 50 µl of DNase I working solution to the centre of the membrane and leave at room temperature (20-30 °C) for 15 minutes. Apply the solution directly to the membrane; do not let it run onto the O-ring or

column wall.

5. Add 350 µl of Wash Solution 1, centrifuge at 12,000 rpm for 30-60 seconds, discard the flow-through and return the column to the collection tube.

6. Continue with step 5 of the standard procedure.

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