CYRN60-CHANGYU EASYspin Bone microRNA Kit

Catalog number	Package
CYRN6001	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 rapid extraction of microRNA from bone tissue, or separate extraction of microRNA and total RNA.

Kit components

Component	Storage	50 preps (CYRN6001)
Lysis Buffer CLB	15-25 °C	50 ml
PLANTaid	15-25 °C	5 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
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. Bone tissue is hard, bone cell density is low, and the surrounding matrix contains large amounts of mucin (proteoglycan) that is difficult to separate from RNA, so conventional Trizol methods do not give high-quality RNA/microRNA. This kit uses a phenol- and chloroform-free route. A dedicated lysis buffer rapidly lyses cells and inactivates cellular RNases; PLANTaid binds proteoglycans and other impurities, which are removed by centrifugation; 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. A separate procedure can enrich the microRNA fraction and recover total RNA (larger than 200 nt) separately.

Features

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

Fast and simple: a single sample is usually processed within 30 minutes.

PLANTaid effectively binds polysaccharides and other impurities and improves their removal.

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 (4 °C is also acceptable) at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C.

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 CLB 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 RNA Clean Kit (CYRN14; in step 2 change 250 µl of absolute ethanol to 700 µl); (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

Before first use, add the indicated amount of absolute ethanol to Wash Solution 1 and Wash Solution 2/3. Transfer 1 ml of Lysis Buffer CLB to a centrifuge tube (if CLB has precipitated at low temperature, redissolve in a 65 °C water bath) and add 100 µl of PLANTaid. Mix by inversion and prewarm in a 65 °C water bath. Scale up proportionally for multiple samples.

1. Liquid nitrogen grinding

a. Crush the bone tissue with bone forceps and place it in a mortar (bake the mortar at 180 °C for 2 hours). Add liquid nitrogen and grind repeatedly to a fine powder, replenishing liquid nitrogen so that it is always present.

b. Transfer 30-150 mg of powder to the prewarmed CLB tube containing PLANTaid. Vortex vigorously for 30-60 seconds or mix by pipetting until a satisfactory homogenate is obtained (or homogenize with an electric homogenizer for 30 seconds). Bone tissues differ greatly; adjust the sample amount according to the specific bone type and experimental results to obtain better yield and purity.

c. Proceed immediately to step 3.

2. Other bone disruption methods

a. Add 30-150 mg of bone tissue to 1 ml of prewarmed CLB containing PLANTaid and homogenize with a high-speed homogenizer. Alternatively, add 30-150 mg of liquid-nitrogen-frozen, embedded and sectioned bone to CLB containing PLANTaid and homogenize.

b. Proceed immediately to step 3.

3. Return the tube briefly to the 65 °C water bath (10 minutes), inverting once or twice to aid lysis.

4. Centrifuge the lysate at 4 °C at 13,000 rpm for 10 minutes to pellet unlysed debris.

5. Take 500 µl of the supernatant (more may be taken if the capacity of the genomic DNA removal column is not exceeded, which increases yield) and load it onto the DNA removal column placed in a collection tube.

6. Centrifuge immediately at 13,000 rpm for 60 seconds and collect the filtrate, which contains the RNA.

7. 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.

8. 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 13,000 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.

9. 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.

10. 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.

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

12. 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%.

13. 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. Prepare 1 ml of CLB with 100 µl of PLANTaid and prewarm at 65 °C as described above.

1. Prepare the bone homogenate by liquid nitrogen grinding or other disruption as in steps 1-2, then centrifuge at 4 °C at 13,000 rpm for 10 minutes.

2. Take 480 µl of the supernatant (more may be taken if column capacity is not exceeded; reduce the volume if genomic DNA residue is high) into a new tube and add 0.5 volume of absolute ethanol. A precipitate may form; 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 13,000 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 9-13 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 13,000 rpm for 2 minutes. Discard the flow-through.

6. Wash and elute according to standard steps 9-13 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.

❖ 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 8.

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 10 of the standard procedure.

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