CYRN54-CHANGYU EASYspin Plus Bone Tissue RNA Kit

Catalog number	Package
CYRN5401	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Do not store at 4 °C or -20 °C, as precipitation affects performance. Keep all solutions tightly capped after use.

Kit components

Component	Storage	50 preps (CYRN5401)
Lysis Buffer CLB	15-25 °C	50 ml
PLANTaid	15-25 °C	5 ml
Lysis Buffer RLT Plus	15-25 °C	25 ml
Deproteinization Buffer RW1	15-25 °C	35 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
DNA-clear/RNA-bind universal columns and collection tubes	15-25 °C	50 sets

Description

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. This kit uses a dedicated lysis buffer free of phenol, chloroform and β-mercaptoethanol and adds components that remove bone tissue proteoglycans. The DNA-clear/RNA-bind universal column and a new solution system avoid toxic phenol/chloroform and β-mercaptoethanol, and are suitable for efficient extraction of highly pure total RNA from various bone tissues and cells, in as little as 30 minutes. The RNA has high purity and low gDNA residue, is free of protein and other impurities, and is suitable for RT-PCR, RT-qPCR, RNA-seq library preparation, array analysis and other downstream applications.

Features

No toxic phenol, chloroform or β-mercaptoethanol and no ethanol precipitation step.

Simple and rapid: a single sample is usually processed within 30 minutes.

The genomic DNA removal column effectively removes residual gDNA, so the RNA generally requires no DNase digestion and can be used in reverse transcription PCR and real-time PCR.

Broad applicability: suitable for various bone tissues, including mineralized bone.

Repeated column washes ensure high purity; typical A260/A280 is 1.9-2.2 with little residual DNA, suitable for RT-PCR, Northern blot, next-generation sequencing and other applications.

Notes before starting

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

Note: Ethanol and a mortar or other suitable bone-crushing device must be supplied by the user.

Note: Do not exceed the capacity of the DNA-clear/RNA-bind universal column; 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, Lysis Buffer RLT Plus 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: Trace genomic DNA cannot be excluded completely by any total-RNA procedure, and even DNase digestion is not 100% effective. The EASYspin Plus buffer system and genomic DNA removal column remove most DNA, so the RNA generally needs 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, use primer pairs that give different product sizes on genomic DNA and cDNA, treat the RNA with RNase-free DNase I (CYRN45) or perform on-column DNase I digestion before the RW1 wash (CYRN34).

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW as indicated on the label. 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) to shear DNA, reduce viscosity and increase yield. 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, or disrupt 30-150 mg of bone tissue into a fine powder in liquid nitrogen or under frozen conditions according to the homogenizer or grinder manufacturer’s instructions, then add 1 ml of prewarmed 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 or shaking once or twice to aid lysis.

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

5. Transfer the supernatant to a new tube and add half a volume of absolute ethanol (0.5 volume). A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge. If material floats on the surface, pipette through it and take the liquid below.

6. Load the mixture (no more than 750 µl per load; larger volumes can be loaded in two portions) onto a DNA-clear/RNA-bind universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 2 minutes and discard the flow-through. Make sure all liquid has passed through the membrane; if necessary, increase the centrifugal force and time.

7. Place the universal column in a clean 2 ml centrifuge tube (an ordinary clean tube is sufficient; a clean collection tube from a new universal column set can also be used). Add 500 µl of Lysis Buffer RLT Plus to the column, centrifuge at 13,000 rpm for 30 seconds, discard the column and keep the filtrate, which contains the RNA. Add 0.5 volume (250 µl) of absolute ethanol to the filtrate; a precipitate may form but

does not affect the procedure. Mix immediately by pipetting and do not centrifuge.

8. Immediately load the mixture onto a new DNA-clear/RNA-bind universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 2 minutes and discard the flow-through (the RNA is now bound to the column). Return the column to the collection tube.

9. Add 700 μl of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

10. Add 500 μl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Repeat once with another 500 μl of Wash Buffer RW.

11. Return the universal 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.

12. Transfer the universal column to a new 1.5 ml centrifuge tube. Apply 30-50 μl of RNase-free ddH2O to the centre of the membrane (prewarming to 70-90 °C improves yield), leave for 1 minute and centrifuge at 13,000 rpm for 1 minute. Use an elution volume of at least 30 μl; smaller volumes reduce recovery. For a higher RNA concentration, reload the eluate onto the column for a second elution.

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