CYRN44-CHANGYU EASYspin Fibrous Tissue RNA Kit (with DNase I)

Catalog number	Package
CYRN4401	50 preps

Storage and shipping: 15-25 °C, except low-temperature components. Stable for 12 months. Proteinase K is supplied in a ready-to-use glycerol buffer and can be shipped at room temperature; after receipt it is stable for at least 6 months at up to 25 °C, for 12 months at 4 °C and for 2 years at -20 °C. Keep all solutions tightly capped after use.

Kit components

Component	Storage	50 preps (CYRN4401)
Lysis Buffer FLT	15-25 °C	50 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	40 ml
Proteinase K	4 °C	1 ml
DNase Buffer	-20 °C	2.5 ml
RNase-free DNase I	-20 °C	250 μl
RNA spin columns and collection tubes	15-25 °C	50 sets

Description

Fibrous tissues such as skeletal muscle, heart, aorta, esophagus and skin contain large amounts of contractile proteins, connective tissue and collagen, are very difficult to lyse, and often cannot be extracted with conventional Trizol or give degraded RNA. This kit combines Proteinase K digestion with RNase-free DNase digestion in the same workflow, degrading protein and genomic DNA during RNA isolation and giving higher yields of high-quality total RNA. The product is suitable for RNA larger than 200 nt and therefore does not include 5S rRNA, tRNA or other small RNAs; the product represents 15-20% of total cellular RNA. A dedicated lysis buffer with β-mercaptoethanol rapidly lyses cells and inactivates cellular RNases. After dilution, the sample is treated with Proteinase K and centrifuged to pellet debris. Binding conditions are adjusted with ethanol and RNA binds selectively to the silica membrane under high-ionic-strength conditions; DNase is applied on the column to remove residual DNA. Rapid wash and centrifugation steps remove metabolites, proteins and other impurities, and purified RNA is eluted in low-salt RNase-free H2O.

Features

The silica membrane is specially selected; variation in binding capacity between columns is minimal and reproducibility is high.

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

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

Repeated column washes ensure high purity; typical A260/A280 is 1.9-2.2 with little residual DNA, suitable for RT-PCR, RT-qPCR, Northern blot 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, β-mercaptoethanol, a disposable syringe, a mortar and a water bath must be supplied by the user.

Note: Lysis Buffer FLT 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: RNA integrity: run on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes. Because 70-80% of cellular RNA is rRNA, prominent rRNA bands should be visible under UV. On ordinary agarose gels, 28S and 18S appear at about 2 kb and 1 kb, and their positions vary with RNA secondary structure. The 28S band should be 1.5-2.0 times as bright as the 18S band, otherwise the RNA may be degraded. A smear or loss of bands indicates severe degradation. Purity: the highest-quality RNA gives A260/A280 of 2-2.2 in 10 mM Tris, pH 7.5.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW. Before use, add β-mercaptoethanol to Lysis Buffer FLT to a final concentration of 1% (10 µl per 1 ml FLT); prepare fresh, and the prepared FLT can be stored at 4 °C for one month.

1. Disrupt and homogenize thoroughly (very important; otherwise yield is severely reduced). a. Electric homogenization (strongly recommended, highest and most reproducible yield): take about 10-20 mg (less than 30 mg) of fresh tissue, add 300 µl of Lysis Buffer FLT and homogenize thoroughly with a rotor-stator homogenizer (such as TissueRuptor) or a bead mill (such as TissueLyser) according to the manufacturer's instructions. b. Liquid nitrogen grinding and homogenization: grind tissue in liquid nitrogen to a fine powder, transfer 10-20 mg (less than 30 mg) of powder to a 1.5 ml centrifuge tube containing 300 µl of FLT and shake vigorously by hand 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), then transfer the homogenate to a new tube. c. Mortar homogenization: place 10-20 mg (less than 30 mg) of tissue powder in a small mortar, quickly add 300 µl of FLT and grind thoroughly at room temperature. Transfer the homogenate to a new tube. If material loss in the mortar is significant, scale up the starting tissue and FLT proportionally; alternatively, grind in liquid nitrogen and add FLT just as the liquid nitrogen evaporates.

2. Add 590 µl of RNase-free H2O to the homogenate, then add 20 µl of Proteinase K and mix by pipetting.

3. Incubate in a 55 °C water bath for 10 minutes.

4. Centrifuge at room temperature at 13,000 rpm for 3 minutes. A small tissue debris pellet forms, and a small amount of floating material may be visible at the top of the supernatant.

5. Transfer the supernatant to a new 1.5 ml centrifuge tube without carrying over the pellet; keep the pipette tip below the floating material, which may adhere to the outside of the tip.

6. Estimate the supernatant volume accurately and add 0.5 volume of absolute ethanol (usually about 450 µl). A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge.

7. Immediately load the mixture (no more than 720 µl per load; can be loaded in two portions) onto the same spin column placed in a collection tube and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through.

8. Prepare the DNase I working solution: mix 45 µl of DNase Buffer and 5 µl of RNase-free DNase I gently in a new 1.5 ml tube (scale up proportionally for multiple columns) and keep on ice.

9. Add 350 µl of Deproteinization Buffer RW1 to the column, centrifuge at 13,000 rpm for 15 seconds, discard the flow-through and return the column to the collection tube. If on-column DNase digestion is not

required, use 700 µl of RW1 in this step, centrifuge at 13,000 rpm for 15 seconds and proceed directly to step 12.

10. 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.

11. Add 350 µl of Deproteinization Buffer RW1 to the column, centrifuge at 13,000 rpm for 15 seconds, discard the flow-through and return the column to the collection tube.

12. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 15 seconds and discard the flow-through. Repeat once with another 500 µl of Wash Buffer RW.

13. Return the 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.

14. Transfer the column to a new 1.5 ml centrifuge tube. Apply 30-50 µl of RNase-free ddH2O to the centre of the membrane, leave for 2 minutes and centrifuge at 13,000 rpm for 1 minute.

Use an elution volume of at least 30 µl; smaller volumes reduce recovery. To increase RNA concentration, prewarm the RNase-free ddH2O to 90 °C before elution or reload the first eluate onto the column for a second elution.

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