CYRN31-CHANGYU EASYspin FFPE microRNA Kit

Catalog number	Package
CYRN3101	50 preps

Storage and shipping: 15-25 °C. Stable for 9 months at room temperature. Solutions should be clear; if a precipitate forms at low temperature, heat in a 37 °C water bath for a few minutes until clear. Do not store at 4 °C or -20 °C. Proteinase K is supplied as a lyophilized powder for stability and convenient shipping; after receipt, briefly centrifuge the tube and dissolve the powder in 0.5 ml of sterile water (40 mg/ml). Because repeated freeze-thaw cycles reduce activity, aliquot immediately and store at -20 °C. Keep all solutions tightly capped after use.

Applications

For rapid extraction of RNA, including microRNA, from formalin-fixed paraffin-embedded (FFPE) tissue samples. The RNA can be used directly in reverse transcription PCR and real-time PCR.

Kit components

Component	Storage	50 preps (CYRN3101)
Lysis Buffer PKD	15-25 °C	15 ml
Binding Buffer RBC	15-25 °C	25 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
Proteinase K powder (optional, 40 mg/ml after dissolution)	-20 °C	20 mg
RNase-free H2O	15-25 °C	10 ml
Genomic DNA removal columns and collection tubes	15-25 °C	50 sets
RNA spin columns RA and collection tubes	15-25 °C	50 sets

Description

This kit is designed for rapid extraction of RNA, including microRNA, from formalin-fixed paraffin-embedded tissue. A dedicated lysis buffer with Proteinase K rapidly lyses cells and releases RNA. The lysate is passed through a genomic DNA removal column that retains genomic DNA while RNA flows through. Binding conditions of the filtered RNA are adjusted with ethanol, and RNA binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. Rapid wash and centrifugation steps remove metabolites, proteins and other impurities, and purified RNA is eluted in low-salt RNase-free H2O. The RNA is suitable for reverse transcription PCR, real-time PCR and similar applications.

Features

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

Fast and simple: RNA extraction from a single sample is usually completed within 1 hour.

The dedicated genomic DNA removal column and formulation effectively remove residual genomic DNA; in general the RNA does not require DNase digestion and can be used directly in reverse transcription PCR and real-time PCR.

Repeated column washes ensure high RNA purity for downstream applications.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge, such as an Eppendorf 5415C or equivalent.

Note: Do not exceed the capacity of the genomic DNA removal column or RNA spin column RA, otherwise DNA residue or reduced yield will result. RNA/DNA content differs greatly between tissues: thymus and spleen are DNA-rich, and more than 5 mg exceeds column capacity; COS cells are RNA-rich, and more than 3×10^6 cells exceed column capacity. When establishing conditions for an unfamiliar sample, start with less material, for example no more than two 10 μm paraffin sections, and adjust afterwards.

Note: Lysis Buffer PKD and Binding Buffer RBC 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 plasticware and tips, bake glassware at 150 °C for 4 hours and soak plasticware in 0.5 M NaOH for 10 minutes followed by thorough rinsing and sterilization, and prepare solutions with RNase-free water (add DEPC to 0.1% v/v, leave at 37 °C overnight and autoclave).

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, so the RNA 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 or primer pairs that give different product sizes on genomic DNA and cDNA.

Note: RNA integrity: run on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes. Formalin fixation and paraffin embedding cross-link RNA to proteins and cause RNA fragmentation, so a diffuse smear is normally seen under UV, and samples stored longer show more degradation, sometimes with a broad band around 100 bp. This is normal for FFPE RNA. Purity: high-quality RNA gives A260/A280 of 1.8-2.1 in 10 mM Tris, pH 7.5; readings in water may be 1.5-1.9. Concentration: (ng/μl) = A260 × dilution factor n × 40.

❖ Procedure

Read the notes before starting. Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW.

1. Trim excess paraffin from the outside of the block and cut sections 10-20 μm thick. Sections must be at least 10 μm thick; thinner sections fragment the cells and cause microRNA loss during deparaffinization.

2. Collect sections with a total thickness of no more than 40 μm into a 1.5-2 ml centrifuge tube (for example two 20 μm or four 10 μm sections). Alternatively, up to 80 μm total thickness may be used in a 2 ml centrifuge tube (see the scale notes in steps 9, 11 and 13).

3. Add 1 ml of 100% xylene and vortex for 10 seconds. Centrifuge briefly so that all tissue is immersed in xylene.

4. Incubate in a 50 °C water bath for 3 minutes to melt the paraffin, then centrifuge at maximum speed at 20-25 °C for 2 minutes to collect the tissue at the bottom.

5. Carefully remove the xylene supernatant with a pipette without disturbing the pellet.

6. Add 1 ml of absolute ethanol, vortex and centrifuge at maximum speed for 2 minutes. Carefully remove the ethanol supernatant.

7. Add 1 ml of absolute ethanol and repeat step 6 once, removing as much ethanol as possible.

8. Air-dry at room temperature or 37 °C for 10 minutes or until all ethanol has evaporated. Complete removal of ethanol is important; trace ethanol reduces RNA yield.

9. Resuspend the tissue pellet thoroughly in 150 μl (for ≤40 μm total thickness) or 240 μl (for ≤80 μm total thickness) of Lysis Buffer PKD by pipetting or vortexing. Centrifuge briefly, add 10 μl of Proteinase K and mix by pipetting.

10. Incubate at 55 °C for 15 minutes, then at 80 °C for 15 minutes. After the 55 °C incubation, remove the tube to room temperature and reinsert it only after the water bath has reached 80 °C; incubate precisely for 15 minutes, since even a 2-minute extension can cause partial RNA degradation.

11. Add 320 µl (for ≤40 µm) or 500 µl (for ≤80 µm) of Binding Buffer RBC and mix thoroughly by pipetting to adjust binding conditions.

12. Immediately load the mixture onto a genomic DNA removal column placed in a collection tube and centrifuge at 14,000 rpm for 30 seconds. Keep the flow-through, which contains the RNA. Avoid pipetting large undigested clumps onto the column, as they may clog it.

13. Add 1120 µl (for ≤40 µm) or 1750 µl (for ≤80 µm) of absolute ethanol to the flow-through and mix immediately by pipetting; do not centrifuge. When adding 1750 µl of ethanol, first transfer the flow-through to a tube with a capacity greater than 3 ml.

14. Immediately load the mixture (no more than 700 µl per load; larger volumes can be loaded in several portions) onto RNA spin column RA placed in a collection tube. Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

15. Add 500 µl of Wash Buffer RW (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 Buffer RW.

16. Return column RA 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.

17. Remove column RA and place it in an RNase-free centrifuge tube. Apply 30 µl of RNase-free water to the centre of the membrane (prewarming to 70-90 °C improves yield), leave at room temperature for 1 minute and centrifuge at 12,000 rpm for 1 minute. A larger elution volume increases elution efficiency; reduce the volume if a higher RNA concentration is needed, or reload the eluate onto column RA and elute again.

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