CYRN06-CHANGYU EASYspin Plus Blood RNA Kit

Catalog number	Package
CYRN0601	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months. Do not store at 4 °C or -20 °C: low-temperature storage can cause precipitation of the buffers.

Description

This kit isolates total RNA from fresh anticoagulated whole blood. Red blood cells are first selectively lysed to recover leukocytes; RNA is then purified on a DNA-clear/RNA-bind universal spin column with a dedicated buffer system. No phenol, chloroform or β-mercaptoethanol is required, and no ethanol precipitation step is needed.

The resulting total RNA is free of protein and other contaminants and carries little genomic DNA, so it is suitable for RT-PCR, RT-qPCR, RNA-seq library preparation and array analysis. The fastest protocol takes about 8 minutes.

Kit components

Component	Storage	50 preps (CYRN0601)
10× Red Blood Cell Lysis Buffer RLB	15-25 °C	25 ml
Lysis Buffer RLT Plus	15-25 °C	30 ml
Wash 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	100 sets

Before first use add the volume of absolute ethanol indicated on the Wash Buffer RW label and tick the box on the label. Dilute the 10× Red Blood Cell Lysis Buffer RLB to 1× with DEPC-treated water before use.

Features

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

Optimised red blood cell lysis buffer gives fast and complete lysis.

The universal column clears genomic DNA in about 1 minute; in most samples no DNase digestion is needed before RT-PCR or RT-qPCR.

Typical 28S:18S ratio in agarose gels is greater than 2:1.

Repeated column washes give typical A260/A280 ratios of 2.1-2.2.

Notes before starting

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

Note: Lysis Buffer RLT Plus and Wash Buffer RW1 contain irritants. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: Residual genomic DNA cannot be excluded completely by any total-RNA procedure. Where downstream work is sensitive to trace DNA, treat the RNA with RNase-free DNase I or perform on-column DNase I digestion.

❖ Procedure

1. Mix 1 volume of fresh anticoagulated blood (up to 1.5 ml) with 3 volumes of 1× Red Blood Cell Lysis Buffer RLB in an RNase-free tube. Invert to mix; flick the tube if needed.

2. Incubate at room temperature for 10 minutes, inverting gently several times during incubation. If RNA degradation is a concern, lyse on ice and extend the time.

3. Centrifuge at 13,000 rpm for 20 seconds and discard the red supernatant as completely as possible without disturbing the white leukocyte pellet.

4. Loosen the leukocyte pellet by vortexing or flicking, then add 350 µl Lysis Buffer RLT Plus (for less than 0.5 ml blood) or 600 µl (for 0.5-1.5 ml blood). Mix by pipetting and vortex vigorously for 20 seconds. For samples with a high leukocyte count, use 350 µl for up to 2×10^6 leukocytes or 600 µl for 2×10^6-1×10^7 leukocytes.

5. Homogenise by pipetting or vigorous vortexing until a uniform lysate is obtained (or homogenise with an electric homogeniser for 30 seconds) to shear DNA and reduce viscosity.

6. Transfer the lysate to a DNA-clear/RNA-bind universal column placed in a 2 ml collection tube. Centrifuge at 13,000 rpm for 1 minute. Discard the column and keep the flow-through, which contains the RNA.

7. Add 0.5 volume of absolute ethanol to the flow-through and mix by pipetting. A precipitate or haze after ethanol addition is normal; mix immediately and do not centrifuge.

8. Transfer the mixture to a new universal column in a collection tube. Stand for 1 minute, centrifuge at 13,000 rpm for 30 seconds, discard the flow-through and return the column to the tube.

9. Add 700 µl Wash Buffer RW1, stand at room temperature for 30 seconds, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

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

11. Repeat step 10 once.

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

13. Transfer the column to a new 1.5 ml tube. Apply 30-50 µl RNase-free ddH2O to the centre of the membrane, stand for 1 minute, centrifuge at 13,000 rpm for 1 minute. An elution volume below 30 µl reduces recovery. Pre-warming the water to 70-90 °C or reloading the first eluate increases the RNA concentration.

14. Use the RNA directly in downstream applications or store at -85 °C to -65 °C.

Notes

Note: For already separated PBMC or sorted cells, start from step 4.

Note: For samples with very few cells or low RNA content, the CYRN68 EASYspin Plus Ultra-Micro RNA Kit uses a low-binding micro column and is recommended to maximise concentration.

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