CYRN46-CHANGYU Serum/Plasma microRNA Kit

Catalog number	Package
CYRN4601	50 preps

Storage and shipping: 15-25 °C, except Lysis Buffer (4 °C, protected from light). Stable for 6 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 purification of cell-free total RNA, including miRNA, from human and animal plasma and serum.

Kit components

Component	Storage	50 preps (CYRN4601)
Lysis Buffer	4 °C, protected from light	50 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
RNase-free spin columns RA and collection tubes	15-25 °C	50 sets

Description

Research on RNA interference and regulatory small RNAs requires efficient recovery of RNAs about 15-30 nucleotides in size, including siRNA and miRNA. Conventional silica membranes bind such small RNAs poorly, and phenol/guanidinium extraction and isopropanol or ethanol precipitation do not efficiently recover them; serum and plasma are especially difficult. This kit uses a dedicated lysis buffer that directly lyses serum/plasma and inactivates RNases, followed by strong organic extraction to remove proteins and DNA. RNA, including small RNAs, binds to a specially selected silica membrane in high ethanol concentration; special wash buffers remove metabolites, proteins and other impurities, and purified RNA is eluted in a low-salt buffer.

Features

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

No isopropanol or ethanol precipitation step that would lose small RNAs.

A dedicated lysis formulation can process larger serum/plasma sample volumes.

Repeated column washes ensure high purity for RNAi, RT-PCR, Northern blot and other applications.

Notes before starting

Note: Before first use, add the indicated amount of ethanol to Wash Solution 1 and Wash Solution 2/3 and tick the boxes to avoid adding ethanol twice.

Note: Unless otherwise stated, all centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C.

Note: Ethanol and chloroform must be supplied by the user.

Note: Lysis Buffer 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: Because the RNA content of serum/plasma is very low, below the lower limit of spectrophotometric measurement, A260 and A260/A280 readings cannot reliably indicate purity or concentration; yield is best assessed by downstream real-time RT-PCR. Cell-free serum/plasma RNA is mainly smaller than 100 nt, so conventional gel electrophoresis is not suitable for assessing integrity.

❖ Procedure

Read the notes before starting. Before first use, add the indicated amount of ethanol to Wash Solution 1 and Wash Solution 2/3.

1. For each 250 µl of serum or plasma, add 750 µl of Lysis Buffer and vortex or pipette several times to mix and help lysis. For samples with high contamination such as high-protein or high-lipid samples, reduce the sample volume and make up the difference with RNase-free H2O; the final volume ratio of Lysis Buffer to liquid sample is always 3:1 (for example 200 µl sample + 50 µl RNase-free H2O + 750 µl Lysis Buffer).

2. Vortex vigorously and incubate at 15-30 °C for 5 minutes.

3. Add 200 µl of chloroform per 750 µl of Lysis Buffer, vortex vigorously for 15 seconds and leave at room temperature for 2 minutes.

4. Centrifuge at 4 °C at 12,000 rpm for 10 minutes. The sample separates into three layers: lower organic phase, interphase and upper colorless aqueous phase containing RNA. The aqueous phase is about 70% of the Lysis Buffer volume. Carefully transfer the supernatant to a new tube and measure its volume accurately.

5. Add 1.5 volumes of absolute ethanol (at room temperature) and vortex to mix. A precipitate may form but does not affect the procedure; mix immediately by pipetting and do not centrifuge. Proceed immediately to the next step.

6. 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 12,000 rpm for 30-60 seconds. Discard the flow-through.

7. 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.

8. 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.

9. 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.

10. Remove column RA and place it in an RNase-free centrifuge tube. Apply 30-40 µl of RNase-free water to the centre of the membrane (prewarming to 100 °C improves elution), 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 and concentration. If a higher concentration is needed, the elution volume can be reduced to as little as 15 µl, although small volumes reduce yield somewhat.

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