CYRN08-CHANGYU EASYspin Bacterial RNA Kit

Catalog number	Package
CYRN0801	20 preps
CYRN0802	50 preps

Storage: 15-25 °C. Lysozyme is supplied separately and must be stored at 4 °C. Stable for 12 months. If a solution forms a precipitate at low temperature, warm it at 37 °C for a few minutes until clear before use.

Description

The kit rapidly isolates total RNA from bacteria. A dedicated lysis buffer lyses the cells and inactivates cellular RNases; after adjustment of binding conditions with ethanol, RNA binds selectively to a silica membrane in a spin column under high-salt conditions. Fast wash and centrifugation steps remove metabolites and protein, and purified RNA is eluted in low-salt RNase-free water.

Kit components

Component	Storage	20 preps (CYRN0801)	50 preps (CYRN0802)
TE (pH 8.0)	15-25 °C	6 ml	6 ml
Lysozyme	4 °C	20 mg	20 mg
Lysis Buffer RLT	15-25 °C	20 ml	50 ml
Wash Buffer RW1	15-25 °C	15 ml	40 ml
Wash Buffer RW (add ethanol before first use)	15-25 °C	5 ml	10 ml
RNase-free H2O	15-25 °C	10 ml	10 ml
RNase-free spin columns RA and collection tubes	15-25 °C	20 sets	50 sets

Features

The silica membrane gives low column-to-column variation and reproducible recovery.

No toxic phenol or chloroform and no ethanol precipitation step.

A single sample is usually processed within 30 minutes.

Repeated washes give typical A260/A280 ratios of 1.9-2.0 with essentially no DNA carry-over; suitable for RT-PCR, Northern blotting and related applications.

Notes before starting

Note: Add the indicated volume of absolute ethanol to the Wash Buffer RW bottle before first use and tick the box on the label.

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

Note: Ethanol is required but not supplied.

Note: Lysis Buffer RLT 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: To prevent RNase contamination, change gloves frequently, use RNase-free plastics and tips, bake glassware at 150 °C for 4 hours or soak plasticware in 0.5 M NaOH for 10 minutes, and prepare solutions with DEPC-treated water.

Note: Trace DNA cannot be excluded completely by any total-RNA procedure. For quantitative mRNA analysis by qPCR, use intron-spanning primers, primer pairs that give different product sizes on genomic DNA and cDNA, or treat the RNA with RNase-free DNase I; on-column DNase I digestion before the RW1 wash is also possible.

❖ Procedure

Note: Before starting, prepare TE (10 mM Tris-HCl, 1 mM EDTA) containing 1 mg/ml lysozyme or lysostaphin.

1. Collect 1-2 ml of bacterial culture (10^8-10^9 cells) by centrifugation in a 1.5 ml tube and remove the supernatant as completely as possible; residual supernatant must not exceed 20 µl per 100 µl TE used in step 2.

2. Resuspend the cells in 100 µl TE (up to 5×10^8 cells) or 200 µl TE (5×10^8-7.5×10^8 cells) containing 1 mg/ml lysozyme or lysostaphin, or resuspend in TE and add a small amount of lysozyme with a clean tip.

3. Incubate at 15-25 °C for 5 minutes with lysozyme, or at 37 °C for 15 minutes with lysostaphin, to digest the cell wall; vortex for 10 seconds every 2 minutes. Gram-negative bacteria are usually lysed by these conditions and the step may even be omitted, whereas some Gram-positive strains require a higher lysozyme concentration, lysostaphin, glass beads, proteinase K digestion or a combination, adjusted to the strain.

4. Add 350 µl Lysis Buffer RLT (when 100 µl TE was used) or 700 µl (when 200 µl TE was used), mix by pipetting and vortex vigorously for 20 seconds. If visible clumps or insoluble material remain, centrifuge at 13,000 rpm for 3 minutes and transfer the supernatant to a new tube.

5. Add 250 µl 96-100% ethanol (100 µl TE / 350 µl RLT scale) or 500 µl (200 µl TE / 700 µl RLT scale) and mix immediately by pipetting.

6. Load the mixture (not more than 700 µl at a time) onto a spin column RA in a collection tube and centrifuge at 13,000 rpm for 60 seconds; discard the flow-through.

7. Add 700 µl Wash Buffer RW1, stand at room temperature for 30 seconds and centrifuge at 12,000 rpm for 30 seconds; discard the flow-through. If DNA carry-over is visible, extend the RW1 incubation to 5 minutes before centrifugation.

8. Add 500 µl Wash Buffer RW (check that ethanol has been added) and centrifuge at 12,000 rpm for 30 seconds; discard the flow-through. Repeat once.

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

10. Transfer the column to an RNase-free tube and apply 30-50 µl RNase-free water to the centre of the membrane (pre-warmed to 70-90 °C for better recovery). Stand for 1 minute and centrifuge at 12,000 rpm for 1 minute.

11. If more than 30 µg of RNA is expected, repeat step 10 and combine the eluates (higher yield, lower concentration), or reload the first eluate onto the column (higher concentration). A second elution increases yield by 15-30%.

Checking RNA integrity and purity

Integrity: run the RNA on a 1.2% agarose gel in 0.5× TBE at 150 V for 15 minutes and view under UV. Animal rRNA appears at about 5 kb (28S) and 2 kb (18S); the larger band should be 1.5-2.0 times as

bright as the smaller one. A smear or loss of the bands indicates degradation. Purity: A260/A280 of 1.8-2.1 in 10 mM Tris pH 7.5 indicates high-quality RNA. The ratio depends on the pH of the solution: the same RNA may read 1.5-1.9 in water without being impure. Concentration: RNA (ng/μl) = A260 × dilution factor × 40.

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