CYRN43-CHANGYU EASYspin Plus Bacteria/Tissue/Cell RNA Kit

Catalog number	Package
CYRN4301	50 preps

Storage and shipping: 15-25 °C; store lysozyme at 4 °C. Stable for 12 months. Keep all solutions tightly capped after use.

Applications

For rapid extraction of RNA from bacteria, tissues and cells. The dedicated genomic DNA removal column effectively removes residual gDNA, so the RNA can be used directly in reverse transcription PCR and real-time PCR.

Kit components

Component	Storage	50 preps (CYRN4301)
TE Buffer (pH 8.0)	15-25 °C	10 ml
Lysozyme	4 °C	30 mg
Lysis Buffer RLT Plus	15-25 °C	25 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	5 ml
DNA-clear/RNA-bind universal columns and collection tubes	15-25 °C	100 sets

Product update: the genomic DNA removal column and the RNA spin column are the same spin column and are now called DNA-clear/RNA-bind universal columns. The old protocol used an equal volume of 70% ethanol; the current protocol uses 0.5 volume of absolute ethanol, so the kit no longer includes a 70% ethanol bottle.

Description

Building on the EASYspin phenol- and chloroform-free RNA extraction technology, this kit adds a DNA-clear/RNA-bind universal column and a new solution system, generally eliminating the need for DNase digestion and avoiding toxic phenol/chloroform and β-mercaptoethanol. It is suitable for efficient extraction of highly pure, high-quality RNA from a wide range of bacteria, tissues and cells. The RNA has high purity and low gDNA residue, is free of protein and other impurities, and is suitable for RT-PCR, RT-qPCR, RNA-seq library preparation, array analysis and other downstream applications.

Features

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

The DNA-clear/RNA-bind universal column removes gDNA in a 1-minute filtration step without DNase digestion, so the RNA can be used directly in reverse transcription PCR, real-time PCR and high-throughput sequencing library preparation; the fastest protocol takes only about 10 minutes.

Excellent RNA integrity: the typical 28S:18S ratio on agarose gels is greater than 2:1.

Repeated column washes ensure high purity; typical A260/A280 is 2.1-2.2.

Suitable for almost all bacterial species, including difficult strains such as Staphylococcus aureus, Streptomyces, Bacillus thuringiensis and Mycobacterium tuberculosis (consult technical support).

Notes before starting

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

Note: Lysis Buffer RLT Plus 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: The kit removes most DNA residue and the purified RNA generally requires no DNase I treatment. If the downstream application is very sensitive to trace DNA, treat the RNA with DNase I (CYRN45) or perform on-column DNase digestion during extraction (CYRN34).

Note: Do not exceed the capacity of the DNA-clear/RNA-bind universal column; otherwise DNA residue or reduced yield will result. When establishing conditions for an unknown sample, start with less material and adjust afterwards.

Note: The kit can also extract RNA from tissues and cells; follow the protocol of the CYRN28 EASYspin Plus Tissue/Cell RNA Kit, which is available on request or from the company website.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer RW. For bacterial RNA, prepare TE Buffer containing lysozyme or Lysostaphin at a final concentration of 1 mg/ml.

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

2. According to cell type and number, resuspend the cells thoroughly in 100 µl (5×10^8 cells) or 200 µl (5×10^8-7.5×10^8 cells) of TE Buffer containing lysozyme at 1 mg/ml; alternatively, resuspend in TE and add a small amount of lysozyme with a clean tip. For Staphylococcus aureus, add Lysostaphin to a final concentration of 1 mg/ml.

3. Incubate at room temperature (15-25 °C) for 5 minutes with lysozyme, or at 37 °C for 15 minutes with Lysostaphin, to break the cell wall. Vortex for 10 seconds every 2 minutes. Different bacteria differ in the ease of cell wall disruption: the conditions above are sufficient for Gram-negative bacteria such as E. coli and the step may even be omitted; some Gram-positive bacteria such as B. subtilis require lysozyme at 15 mg/ml and 15 minutes. For Staphylococcus aureus, add Lysostaphin to 1 mg/ml and incubate at 37 °C for 15 minutes. Adjust enzyme type, concentration, temperature and time for difficult strains, and combine with glass bead beating, mechanical disruption or Proteinase K digestion if needed.

4. Centrifuge briefly for 1 minute to collect the cells, remove the supernatant and vortex to resuspend and disperse the cells.

5. Add 500 µl of Lysis Buffer RLT Plus, mix by pipetting and vortex vigorously for 20 seconds. If obvious clumps or insoluble material remain, centrifuge at 13,000 rpm for 3 minutes and transfer all of the supernatant to a new tube.

6. Load the homogenate onto a DNA-clear/RNA-bind universal column placed in a 2 ml collection tube and centrifuge at 13,000 rpm for 1 minute. Discard the column and keep the filtrate, which contains the RNA.

7. Add 0.5 volume of absolute ethanol (about 250 µl) to the filtrate and mix by pipetting. Cloudiness or flocculation after adding ethanol is normal; mix immediately and do not centrifuge. If you prefer the old protocol with an equal volume of 70% ethanol, add 500 µl of 70% ethanol instead (prepare 70% ethanol as 9 ml RNase-free H2O + 21 ml absolute ethanol).

8. Immediately load the mixture onto a new DNA-clear/RNA-bind universal column placed in a collection tube. Leave for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Return the column to the collection tube.

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

10. 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.

11. Repeat step 10 once.

12. Return the universal 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.

13. Transfer the universal column to a new 1.5 ml centrifuge tube. Apply 30-50 μl of RNase-free H2O to the centre of the membrane, leave for 1 minute 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 H2O to 70-90 °C before elution or reload the first eluate onto the column for a second elution.

14. The extracted RNA can be used directly in downstream applications or stored at -85 to -65 °C.

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