Ver. 24122

ODP400

Bacteria RNA Kit

For extraction of RNA from bacteria

ISO 13485:2016 ISO 9001:2015

Bacteria RNA Kit

Spin Column

Kit Contents

Contents	50 Preps
Buffer RZ	50mL
Buffer RD	13mL
Buffer RW	15mL
Buffer LZ	10mL
Lysozyme	1mL
RNase-free Water	10mL
Spin column CR3	50
Collection Tubes (2mL)	50

Storage

Buffer RZ

For short term (≤1 month): At room temperature, protected from light

For long term (≥1 month): At 2-8°C, protected from light

Other reagents: At room temperature

Note: Buffer RZ may be transported at room temperature

Introduction

Bacterial RNA Kit uses a new technology based on guanidine thiocyanate/phenol method. It contains a unique Buffer RZ that minimizes the contamination of genomic DNA and protein. Bacterial RNA Kit can efficiently extract high pure RNA from bacteria in one hour. The extracted RNA is ready-to-use in downstream applications such as: PCR, gene chips assay, northern blot, dot blot, poly A screening, *in vitro* transcript and molecular cloning.

Notes for avoiding RNase contamination

- Change gloves regularly.
- Use RNase-free plasticwares or glasswares to avoid cross contamination.
- To remove RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5M NaOH for 10 minutes, washed by RNase-free Water thoroughly and sterilized.
- Use RNase-free Water. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 minutes to remove any trace of DEPC).

Protocol

Buffer RW are supplied as a concentrate. Before use, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

1. Pipette 1-5mL bacterial culture suspension in a microcentrifuge tube by centrifuging for 5 minute at 5,000 rpm. Discard supernatant.
2. Add 1 mL Buffer RZ, mix thoroughly by vortexing.
   Note: For Gram positive bacteria, use Lysozyme (50mg/mL) (Cat. No.: ORT401-01) for lysis.
   - **Add 180µL Buffer LZ to the bacterial pellet, followed by 20µL Lysozyme.**
   - **Incubate for at least 30 minutes at 37°C. Add 1mL Buffer RZ to the tube and mix the contents by repeated pipetting.**
3. Incubate homogenized samples at 15-30°C for 5 minutes, to permit complete dissociation of the nucleoprotein complex.
   Optional step: Centrifuge the sample at 12,000 rpm (~13,400 ×g) for 10 minutes at 4°C. Transfer the supernatant to a fresh microcentrifuge tube.
4. Add 200µL of chloroform per 1mL Buffer RZ used for homogenization. Cap the tube securely and mix the sample by inverting the tube for 15 seconds. Incubate for 3 minutes at room temperature.
5. Centrifuge the sample for 10 minutes at 12,000 rpm (~13,400 ×g) at 4°C. The mixture separates into a lower pink phenol chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase out into a new tube.
   Note: Precipitates are expected, but it will not interfere with the extraction.
6. Add the 0.5 volume ethanol (96%-100%) to the aqueous phase. Mix thoroughly (precipitate may appear in this step).
   Note: Add 0.5mL ethanol if the initial volume of Buffer RZ is 1mL.

7. Transfer the sample from step 6, including any precipitate that may have formed, to an RNase- free spin column CR3 placed in a 2mL RNase-free collection tube. Close the lid gently, incubate at room temperature for 5-10 minutes and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
   Note: Since the capacity of CR3 is 700µL, the loading-centrifugation step must be repeated for processing all the mixture from step 6.
8. Add 500µL Buffer RD to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
9. Add 700µL Buffer RW to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds at 4°C. Discard the flow- through.
10. Repeat step 8.
    Note: A second wash with 700µL PW is recommended, if the salts have not been completely removed from the spin column.
11. Set the RNase-free spin column CR3 back to the collection tube. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes at 4°C to dry the spin column membrane. Open the lid of the spin column CR3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.
    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
12. Place the RNase-free spin column CR3 in a new 1.5mL RNase-free microcentrifuge tube. Add 30-100µL RNase-free Water directly to the spin column membrane. Close the lid gently, and incubate at room temperature (15–25°C) for 2 minutes. Centrifuge at 12,000 rpm (~13,400 × g) for 2 minutes at 4°C to elute the RNA.
    Note: The volume of elution buffer should not be less than 30µL, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 13 to the center of membrane again, let the columns stand for 1 minute, and then centrifuge. Extracted RNA should be stored at –80°C.