

Ver. 24082

ODP400

Bacterial RNA Kit

For extraction of RNA from bacteria

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ISO 13485:2016 ISO 9001:2015

Bacterial RNA Kit

(Spin Column)
Cat. No. ODP400

Kit Contents

Contents	50 Preps
Buffer RZ	50mL
Buffer RD	13mL
Buffer RW	15mL
DNase I (RNase-free)	1500U
Buffer RDD	4mL
RNase-free Water	10mL
RNase-free spin column CR3 & Collection tube (2mL)	50
Hand book	1

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

Note: It is advised to reconstitute ONLY required volume of Buffer RD with ethanol (96-100%) as reconstituted Buffer RD will precipitate on long term storage.

1. Pipette 1-5mL bacterial culture suspension in a microcentrifuge tube by centrifuging for 1 minute at 10,000 rpm ($\sim 11,500 \times g$). 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, not supplied) for lysis.

- Add 180µL Buffer LZ (not supplied) 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.

Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clear aqueous phase part for next step.

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.
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 5.

8. Add 80µL DNase I working solution to the center of Spin Column CR3, and incubate at room temperature for 15 minutes.

- **Preparation of DNase I storage solution: Dissolve the DNase I powder (1500 U) in 550µL 0.15M NaCl, gently mix well, and store in -20°C (for up to 9 months) after aliquoting.**

Note: The thawed the DNase I storage solution from -20°C can be stored at 4 °C (for up to 6 weeks). Do not freeze again.

- **Preparation of DNase I working solution: Add 10µL of DNase I storage solution into a new RNase-free centrifuge tube, then add 70µL Buffer RDD and mix well gently (gently mix with a pipette).**

9. 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.
10. 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.
11. Repeat step 10.

12. Set the RNase-free spin column CR3 back to the collection tube. Centrifuge at 12,000 rpm ($\sim 13,400 \times g$) 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.

13. 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 ($\sim 13,400 \times g$) 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.