

## Product information

# Rapid Bacteria RNA Isolation Kit

**Catalog #:** BS8625  
**Size:** 50 Prep  
**Storage:** 4°C

### About:

This kit is designed for preparation of high quality total RNA from bacteria cells. 20 µg total RNA can be purified from  $5 \times 10^7$  bacteria cells using this kit. Purified RNA is ready for most downstream applications such as RT-PCR, Northern Blotting, Poly (A) purification, nuclease protection and in vitro translation.

### Contents:

Description	Size
Buffer Rlysis-B	50 ml
RNase-free Water	5 ml
Protocol	1

### Materials Supplied by User:

- Microcentrifuge capable of at least 12,000 × g.
- RNase-Free pipets and pipet tips.
- Vortexer.
- RNase-Free Ethanol (96-100%).
- RNase-Free Microcentrifuge tubes (1.5 ml or 2 ml).

### Features:

- Fast. Using fast lysis buffer, the whole procedure takes less than 40 minutes.
- High quality of RNA. Purified RNA has an OD<sub>260</sub>/OD<sub>280</sub> ratio of 1.9-2.0.
- Easy to scale up.

**NOTE:** Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all times.

### Procedure:

#### 1. Sample Preparation.

##### A. Gram-negative bacteria (E. coli, streptococcal, pneumococcal, etc.):

- a. Transfer logarithmic phase culture (about  $2 \times 10^9$  cells) into centrifuge tube and centrifuge at 10,000 × g for 30 seconds, discard supernatant.
- b. Add 100 µl lysozyme solution (400 µg/ml lysozyme in RNase-free Water. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 5 minutes.

## Procedure (contd.):

### 1. Sample Preparation:

#### B. Gram-positive bacteria (golden staphylococcal, orynebacteriadiphtheriae, etc.):

- a. Transfer logarithmic phase culture (about  $2 \times 10^9$  cells) into centrifuge tube and centrifuge at  $10,000 \times g$  for 30 seconds, discard supernatant.
- b. Add 100  $\mu$ l lysozyme solution (3 mg/ml lysozyme in RNase-free Water. NOT supplied in the kit), suspend thoroughly and incubate at  $37^\circ\text{C}$  for 5 minutes.

2. Using RNase-free pipet tips, add 1 ml Buffer Rlysis-B and mix by inverting immediately.

3. Incubate at room temperature for 5 minutes to make sure the cells are completely lysed.

4. Add 200  $\mu$ l chloroform to the tube, mix by inverting.

5. Centrifuge at  $12,000 \times g$  for 5 minutes at  $4^\circ\text{C}$ . Transfer the supernatant to a new RNase-free 1.5 ml tube.

6. Add 1/3 volume of ethanol to the tube, vortex for 30 seconds.

**Note:** Incubating at  $-20^\circ\text{C}$  for 5-10 minutes can improve the RNA yield.

7. Centrifuge at  $12,000 \times g$  for 5 minutes at  $4^\circ\text{C}$ , discard the supernatant carefully.

8. Add 1 ml of RNase-free 75% ethanol to the tube, mix well by inverting 10 times.  
Centrifuge at  $12,000 \times g$  for 1 minute, discard the supernatant.

9. Repeat the Step 8 once.

10. Air-dry the pellet at room temperature with the lid open for 2~5 minutes.

**Note:** Don't over dry. This step is very important, residual ethanol in RNA will interfere some downstream applications.

11. Add 30~50  $\mu$ l of RNase-free Water to dissolve RNA pellet. The purified RNA is ready for use, or keep at  $-70^\circ\text{C}$  for long term storage.



PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.  
NOT INTENDED FOR HUMAN OR ANIMAL USE.