PRODUCT INFORMATION

Rapid Bacteria RNA Isolation Kit

Product information for BS8625:

Kit Contents

Components	BS8625, 50 Preps
Buffer Rlysis-B	50 ml
RNase-free Water	5 ml
Protocol	1

Storage

Store at room temperature. The kit is valid for 1 year at 4°C.

Introduction

This kit is designed for preparation of high quality total RNA from bacteria cells. 20 µg total RNA can be purified from 5 x 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.
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.

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Features

- ü Fast. Using fast lysis buffer, the whole procedure takes less than 40 minutes.
- ü High Quality of RNA. Purified RNA has an OD_{260}/OD_{280} ratio of 1.9-2.0.
- ü Easy to scale up

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)

Procedures

1. Sample Preparation.

A. Gram-negative bacterials (*E. coli*, streptococcal, pneumococcal, *etc.*)

a. Transfer logarithmic phase culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 x *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.

B. Gram-positive bacterial (golden staphylococcal, orynebacteriadiphtheriae, *etc.*)

a. Transfer logarithmic phase culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 *x g* for 30 seconds, discard supernatant.

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b. Add 100 µl lysozyme solution (3 mg/ml lysozyme in RNase-free Water. NOT supplied in the kit), suspend thoroughly and incubate at 37°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 µl chloroform to the tube, mix by inverting.
5. Centrifuge at 12,000 *x g* for 5 minutes at 4°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°C for 5-10 minures can improve the RNA yield.
7. Centrifuge at 12,000 *x g* for 5 minutes at 4°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 *x 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.

    Note1: This step is very important, residual ethanol in RNA will interfere some downstrean applications.

    Note2: Don't over dry.
11. Add 30~50 µl of RNase-free Water to dissolve RNA pellet. The purified RNA is ready for use. Or keep at -70°C for long term storage.

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

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