Vazyme

Vazyme Biotech Co., Ltd

Bacteria RNA Extraction Kit

Cat # R403-01

Introduction

The Bacteria RNA Extraction Kit is a kit optimized for RNA extraction from prokaryotes, including Gram-negative and Gram-positive bacteria. The kit includes a bacterial RNA Plus Reagent containing an optimized buffer system, a chelating agent and a mild protein denaturant, and an RNA isolater Total RNA Extraction Reagent that efficiently inhibits endogenous RNase and promotes protein denaturation. The combination of the two can greatly improve the extraction yield of prokaryotic RNA while ensuring good product quality. When extracting bacterial RNA, a high-temperature pretreatment is added only before the routine operation, and no high-quality and high-yield bacterial total RNA can be obtained without mechanical disruption or enzymatic hydrolysis steps. This kit is also suitable for the extraction of total RNA from fungi such as yeast.

Package Information

Components	R403-01 (100 rxn)
Bacteria RNA Plus Reagent	20 ml
RNA Isolater Total RNA Extraction Reagent	100 ml

Storage

Store RNA Isolater Total RNA Extraction Reagent at 4°C protected from light;

Store Bacteria RNA Plus Reagent at room temperature. If the reagent precipitates during storage, please dissolve and mix at 65 °C for a while.

Advantages

The bacterial RNA extraction kit achieves the following outstanding results:

1. Significantly increase the total RNA extraction amount of Gram-negative bacteria and Gram-positive bacteria;
2. Ensure good RNA product quality, and more easily meet the needs of subsequent experiments;
3. Easy to operate, save time, no need for lysozyme or ultrasound, grinding and other wall breaking treatment, which eliminate the RNA degradation that may be introduced by the operation;
4. No bacterial genomic DNA contamination.

User-preparation

Thermostat water bath/metal bath, refrigerated centrifuge, vortex shaker (vortex mixer), chloroform, isopropanol, 75% ethanol (prepared with DEPC water), RNase-free water/DEPC water; RNase-free tip and RNase-free 1.5 ml tube.

Protocol

Step 1: Preparation of Bacteria RNA Plus Reagent

1. Inoculate the target prokaryotic cells in a suitable medium.

2. Culture the bacteria to the logarithmic phase at the appropriate temperature. Cells that have passed the logarithmic growth phase will not be suitable for RNA extraction and will need to be re-inoculated.

3. Preheat the water bath to 95 °C and pre-cool the refrigerated centrifuge to 4 ° C.

4. Transfer 1 to 1.5 ml of bacterial solution (the number of cells up to 10^8) to a pre-cooled centrifuge tube.

5. Centrifuge the cells at 10,000 g for 3 min at 4 °C.

6. During this period, the required amount of Bacteria RNA Plus Reagent reagent should be preheated in a 95 °C water bath. Do not repeatedly heat the whole bottle, which tends to make the product properties unstable.

7. Remove the supernatant after centrifugation, and resuspend the cells with 200 μl of pre-heated Bacteria RNA Plus Reagent reagent. If the amount of cells is less than 5 x 10^7, use 100 μl of Bacteria RNA Plus Reagent reagent.

8. After rapid resuspension, water bath at 95 °C for 4 min, when the number of cell tubes is large, please strictly control the treatment time. Too short (such as less than 3 min) treatment time may lead to insufficient lyses of the cell, but the treatment time should not exceed 5 min.

9. Add 1 ml of RNA Isolater Total RNA Extraction Reagent to the treated cell suspension, mix by pipetting, and place on ice for 5 min.

Step 2: Total RNA Isolation

1. Add 1/5 lysate volume of chloroform to the above lysate. Tightly cap the tube lid, shake by hand, and vortex for 15 sec into emulsion, and then let it stand on ice or at 4 ° C for 5 min.

2. Centrifuge at 12000 g for 15 min at 4 °C. Note that this step must be centrifuged at low temperature, otherwise the product will be contaminated with a small amount of genomic.

3. Carefully remove the tube. At this point the solution is divided into three layers: a colorless upper layer, a

white intermediate layer, and a red lower layer. Carefully pipette the upper aqueous phase into a new centrifuge tube.

* The upper volume accounts for approximately 60% of the total volume. It is recommended to take 600 μl and do not suck too much to prevent absorbing the middle layer to lead to genomic contamination.

4. Add an equal volume of pre-cooled isopropanol and mix by inverting. Let it stand at -20 ° C for 10 min.

5. Centrifuge at 12000 g for 10 min at 4 °C. A white precipitate is usually visible.

6. Carefully discard the supernatant and add 1 ml of pre-cooled 75% ethanol (prepared with DEPC water). Wash the tube lid and wall thoroughly, and gently flick the bottom of the tube to allow the pellet to float and stand at 4 ° C or ice for 3-5 min.

7. Centrifuge at 12000 g for 5 min at 4 °C. Discard the supernatant.

* To reduce the residual impurities, the supernatant should be discarded as much as possible. It is recommended to discard most of the supernatant, and briefly centrifuge all liquids to the bottom of the tube, and then pipette the remaining liquid.

8. Dry the precipitate for 2-5 min at room temperature in a clean environment. Be careful not to dry excessively, otherwise it will cause RNA to be difficult to dissolve.

9. Add the appropriate amount of RNase-free water (DEPC water) to dissolve the pellet and gently pipette until the pellet is completely dissolved. After that, take a small amount to detect and store the rest at -80 °C.

Step 3: Product Detection

A. Integrity detection

1. Take 1 μl of RNA into 8 μl of TE, add 1 μl of 10 x DNA loading buffer, and mix them.

2. Perform 1% agarose gel electrophoresis. Prokaryotic organisms will show two clear bands of 23S/16S ribosomal RNA, and a weak band of 5S, which proves that RNA integrity is good.

B. Purity and concentration detection

1. Dilute the RNA with TE, and measure the OD values at 230 nm, 260 nm, and 280 nm using a spectrophotometer, and calculate OD_{260}/OD_{280} and OD_{260}/OD_{230}. The OD_{260}/OD_{280} ratio of pure RNA should be between 1.8 and 2.2. If it is less than 1.8, there may be DNA or protein residues; if it is less than 1.6, the RNA may be excessively dried. The ratio of OD_{260}/OD_{230} should be around 2, too low indicates that there is residual organic solvent, and it is not washed enough by 75% ethanol.

2. RNA Concentration (ng/μl) = OD_{260} x dilution factor x 40.

Expected Output

Taking Gram-negative bacteria Escherichia coli as an example: isolate RNA from 1.5 ml logarithmic phase cells (OD600= 1.0, 1.5 x 10^8 cells)using Bacteria RNA Extraction Kit, RNA yield is greater than 30 μg, for Gram-positive bacteria Bacillus subtilis, The RNA yield of the same cell number is greater than 20 μg. The

extracted RNA product was subjected to 1% agarose gel electrophoresis, showing clear 16S and 23S ribosomal RNA bands, the 23S band was slightly deeper, and the 5S ribosomal RNA band was extremely weak.

FAQ and Solutions

Q. RNA production is low.

A. Extraction of prokaryotic RNA using Bacteria RNA Extraction Kit does not achieve the expected yield. The possible reasons are: a. The lysis is not complete enough or the initial bacterial amount is lower than the required starting amount, and the initial bacterial amount can be appropriately increased, or the treatment time can be prolonged up to 5 min; b. RNA precipitation is not completely dissolved, and RNA can be fully dissolved by heating at 60 ° C for 5 min.

Q. RNA degradation

A. Using Bacteria RNA Extraction Kit to treat bacteria for a few minutes does not cause RNA degradation. After adding RNA Isolater, it can fully contact with free RNA to protect RNA from degradation. The process after separating the aqueous phase may cause degradation if RNase is present. Therefore, it is necessary to use RNase-free tips and centrifuge tubes during the experiment, and use DEPC-treated water to prepare reagents, dissolve RNA and ensure the cleanliness of the operating area. Some prokaryotic bacteria, such as Bacillus subtilis, have high levels of endogenous RNase and are difficult to completely inactivate. If the product is partially degraded, the amount of starting bacteria can be reduced, and use 200 µl of treatment solution, and the experiment should be carried out in strict accordance with standard operating procedures. Keep the operation at a low temperature and try to minimize the unnecessary operation time.