Bacteria RNA Enhancement Reagent

R412-C1

Version 24.1

Product Description

Bacteria RNA Enhancement Reagent is designed for RNA extraction from prokaryotes (including Gram-negative and Gram-positive bacteria). Bacteria RNA Enhancement Reagent contains an optimized buffer system, chelating agents, and mild protein denaturants. When used with VeZol Reagent (Vazyme #R411), it can inactivate endogenous RNases and promote protein denaturation, significantly improving the RNA yields while ensuring good product quality. This reagent is also applicable for RNA extraction from yeast, Candida albicans, and other fungal cells.

Components

Components	R412-C1 (100 rxns)
Bacteria RNA Enhancement Reagent	20 ml

Storage

Store at 15 ~ 25°C and ship at room temperature.

Applications

10^7 ~ 10^9 bacterial or fungal cells.

Applicable Instruments

Thermostatic water bath/metal bath, refrigeration centrifugation machine, vortex mixer;

RNase-free pipette tip and RNase-free 1.5 ml centrifuge tube;

VeZol Reagent (Vazyme #R411), chloroform, isopropanol, 75% ethanol (DEPC ddH_2O prepared), RNase free-ddH_2O/DEPC ddH_2O.

Experiment Process

◇ **Bacteria RNA Enhancement Reagent pre-treatment**

1. In suitable culture medium, inoculate the target prokaryotic cells.
2. Under appropriate temperature conditions, shake the bacteria to the logarithmic growth phase of the cells. Cells that have grown beyond the logarithmic growth phase will not be suitable for RNA extraction, and it is recommended to re-inoculate the cells.
3. Preheat water bath to 95°C, pre-cool refrigeration centrifuge to 4°C.
4. Transfer 1 - 1.5 ml bacterial culture (cell count can reach 10^9 level) to centrifuge tube.
5. Centrifuge at 11,200 rpm (12,000 × g) for 3 min to settle cells.
   ▲ During this period, place the required amount of Bacteria RNA Enhancement Reagent into a 95°C water bath for preheating.
   ▲ Please do not repeatedly heat the entire bottle, as it may cause the product properties to become unstable.
6. After centrifugation is complete, discard the supernatant and resuspend the cells by pipetting with 200 µl of pre-warmed Bacteria RNA Enhancement Reagent.
   ▲ If the cell amount is less than 5 × 10^7, 100 µl Bacteria RNA Enhancement Reagent can be used for treatment.
7. Incubate at a 95°C water bath for 4 min.
   ▲ When the number of cell tubes is relatively high, please try to strictly control the processing time. A processing time that is too short (e.g., <3 min) may lead to insufficient cell lysis, but do not exceed 5 min.

8. Add 1 ml VeZol Reagent (Vazyme #R411) to the processed cell suspension, and pipette up and down several times to mix thoroughly, then place on ice for 5 min.

◇ **Total RNA extraction**

1. Add 1/5 volume of chloroform to the above lysis buffer, shake vigorously for 15 sec to form an emulsion, and let it stand at room temperature for 5 min.
2. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 15 min.
3. Carefully take out the centrifuge tubes. At this time, the solution is divided into three layers: upper aqueous phase (containing RNA), middle layer, and red organic layer. Carefully transfer the upper aqueous phase (approximately 500 µl) into a new RNase-free centrifuge. Do not disturb the interphase.
   ▲ The upper layer volume accounts for approximately 60% of the initial VeZol Reagent volume. If 1 ml of VeZol Reagent is used for extraction, the upper aqueous phase is approximately 600 µl.
4. Add equal volume isopropanol, mix by inversion, let it stand at room temperature for 10 min.
5. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 10 min, and a white precipitation will usually be observed. Carefully discard the supernatant.
   ▲ The precipitation will be barely visible for samples with a low RNA content. Proceed with the workflow.
   ▲ To minimize residual impurities, discard supernatant as completely as possible in this step. After removing the supernatant, the RNase-free tube can be left inverted on a clean blotting paper for 1 min. Do not discard or pipette out the RNA precipitation.
6. Add 1 ml 75% ethanol (RNase-free ddH_2O prepared). Gently flick the bottom of the tube to suspend the precipitate, and invert several times. Centrifuge at 11,200 rpm (12,000 × g) 4°C for 5 min. Be careful to discard the supernatant.
   ▲ To minimize residual impurities, discard supernatant as completely as possible in this step. Take care not to lose the precipitation. It is recommended to discard most of the supernatant, spin the tube briefly to collect all the liquid to the bottom, and then remove the remaining liquid with a pipette. Take care not to lose or disturb the RNA precipitation.
7. In a clean environment, dry the precipitate at room temperature for 2 - 5 min, add 20 - 100 µl RNase-free ddH_2O to dissolve the precipitate, vortex at room temperature or repeatedly pipette to resuspend the RNA. The extracted RNA can be aliquoted and stored at -85 ~ -65°C for long-term storage or -30 ~ -15°C for short-term storage.
   ▲ Do not overdry the precipitation, or the RNA will be hard to dissolve.
   ▲ RNA dissolution can be facilitated by incubating in a 55 ~ 60°C water bath for 10 - 15 min.

FAQ & Troubleshooting

◇ Low RNA yields

1. Incomplete lysis or lower bacterial input: the initial bacterial input can be appropriately increased, or the 95°C treatment time can be extended to 5 min.
2. Incomplete dissolution of RNA pellet: the pellet can be heated at 60°C for 5 min to fully dissolve RNA.
3. Bacillus species have a special cell wall, and the combination of lysozyme (Vazyme #DE103) and VeZol Reagent (Vazyme #R411) will get a higher yield.

◇ RNA degradation

1. Using Bacteria RNA Enhancement Reagent with a high temperature treatment for several minutes, it will not cause RNA degradation. After adding VeZol Reagent (Vazyme #R411), the VeZol can fully protect RNA. The seperated RNA in the aqueous phase are easily be degraded if RNase present. Therefore, during the entire experimental process, it's neccessary to use RNase-free pipette tips and centrifuge tubes, prepare reagents and dissolve RNA with DEPC ddH_2O, ensure the cleanliness of the operating area.
2. Certain prokaryotic bacteria such as Bacillus subtilis have a high content of endogenous RNase, making it difficult to completely inactivate. If degradation of the product is observed, the initial bacterial amount can be appropriately reduced, using 200 µl Bacteria RNA Enhancement Reagent. The experiment is carried out in strict accordance with the standard operation process, the whole process is kept at low temperature, and the unnecessary operation time is shortened as far as possible with skilled operation techniques.

◇ Genomic residue

1. Refer to VeZol Reagent (Vazyme #R411) manual.