HiPure Bacterial RNA Mini Kit

Introduction

This kit is suitable for extracting high-purity total RNA from Gram negative or positive bacterial culture media. This kit is based on silica gel column purification technology, providing lysozyme and acidic glass beads, which is suitable for extracting RNA from various bacteria. The extraction does not require toxic phenol chloroform extraction, and only takes 30-40 minutes. The obtained RNA can be directly used for RT-PCR Northern Blot, Poly A purification, nucleic acid protection, and in vitro translation experiments.

Kit Contents

Cat. No.	R418101	R418102	R418103
Purification times	10 preps	50 preps	250 preps
gDNA Filter Mini Columns	10	50	250
HiPure RNA Mini Columns	10	50	250
2ml Collection Tubes	20	100	500
Glass Beads (0.1-0.6mm)	10 g	30 g	150 g
Plastic spoon	2	4	10
Lysozyme	20 mg	90 mg	400 mg
Protease Dissolve Buffer	1.8 ml	1.8 ml	10 ml
Buffer TE	1.8 ml	1.8 ml	5 ml
Buffer STL	5 ml	20 ml	90 ml
Buffer RLC	10 ml	30 ml	150 ml
Buffer RW1	10 ml	50 ml	250 ml
Buffer RW2*	5 ml	20 ml	2 x 50 ml
RNase Free Water	1.8 ml	10 ml	30 ml

Storage and Stability

The kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions. Lysozyme should store at 2-8°C or -20°C after received. RNase Free Water does not contain antibacterial agents, it may be contaminated by bacterial or fungal when placed or operated at room temperature. It is recommended to subpackage and store at 2-8°C to reduce contamination.

Preparation before use

- Preparation of Lysozyme (50mg/ml): Add 0.4ml (10 preps), 1.8ml (50 Preps) or 8ml (250 Preps) Protease Dissolve Buffer to the bottle of Lysozyme and store at 2-8°C or -20°C.
- Add 20ml (10 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) absolute ethanol to the bottle of Buffer RW2 and store at room temperature.
- **Add DTT or 2-mercaptoethanol to improve RNA integrity:** Add 20µl 2-mercaptoethanol (or 20µl 1M DTT) to 1ml Buffer RLC/STL can improve the inactivation effect of nucleases, and the mixture can be placed at room temperature for one week.

Bacterial amount (<1 x 10^9) and Cultivation

- **Bacterial amount:** Bacterial growth can be measured using a spectrophotometer. Due to the difference of different instruments and the influence of various growing conditions, it is difficult to give an accurate and reliable relationship between OD value and the number of bacteria. For example, when a culture medium containing 1 x 10^9 bacteria per milliliter was diluted four times, OD600 was 0.125 when measured by Beckman Du-40 while that was 0.25 when measured by DU-7400. Therefore, we recommend using the plate counting method to adjust the OD value measured by the instrument. When measuring OD, dilute or concentrate the sample to ensure that the reading is within the confidence interval of 0.05-0.3. The RNA content varies greatly among different culture conditions and different bacteria, thus we recommend a bacterial amount of 5 x 10^8 for the initial extraction, and then adjust according to the results.
- **Bacterial cultivation:** Select a suitable culture medium and inoculate bacterial in a 1:100 ratio. Shake at 37°C for 6-10 hours, depending on the growth rate of the bacteria. It is recommended to extract RNA during exponential phase.

Protocol 1 Enzyme method

This protocol is suitable for Gram negative bacteria or some Gram positive bacteria.

1. Take 0.5-1.8ml bacterial culture medium (<1x10^9) in the exponential phase and transfer to a 2.0ml centrifuge tube. Centrifuge at 12,000 × g for 1 minute to collect bacteria. Discard the culture medium and invert it onto an absorbent paper to absorb the remaining liquid.
2. Add 100µl Buffer TE and 10-20µl Lysozyme (50mg/ml), vortex to resuspend the bacteria, place at room temperature for 5-15 minutes to digest the bacterial cell wall.
3. Add 500µl of Buffer RLC/2-mercaptoethanol, vortex for 10 seconds, place at room temperature for 5 minutes, centrifuge at 12,000 × g for 1 minute.
4. Insert a gDNA Filter Mini Column into a 2ml collection tube, transfer 0.6ml supernatant to the column. Centrifuge at 12,000 x g for 1 minute and discard the gDNA filter Mini column.
5. Add 0.18ml isopropanol to the filtrate, pipette and mix for 3-5 times.
6. Insert a HiPure RNA Mini Column into a 2ml collection tube. Transfer all the mixture to the column. Centrifuge at 12,000 x g for 1 minute.
7. Discard the filtrate, insert the column back into the collection tube. Add 500µl Buffer RW1. Centrifuge at 12,000 x g for 1 minute.

- DNA filtration columns can remove 95-99% of genomic DNA contamination. Most applications do not require further processing. Due to the high sensitivity of PCR, single copy gene may also be amplified. If purified RNA is used for RT-PCR, it is recommended to order our DNase on Column Kit (R4911) to completely remove DNA on the membrane.

8. Discard the filtrate, insert the column back into the collection tube. Add 500µl Buffer RW2. Centrifuge at 12,000 x g for 1 minute.
9. Discard the filtrate, insert the column back into the collection tube. Add 500µl Buffer RW2. Centrifuge at 12,000 x g for 1 minute.
10. Discard the filtrate, insert the column back into the collection tube. Centrifuge at 12,000 x g for 2 minutes.
11. Transfer the column to a 1.5ml centrifuge tube and add 30-100µl RNase Free Water to the center of the column membrane. Place at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute. Discard the column and store the RNA at -80℃ or -20℃.
    The minimum elution volume of the column is 30µl. If the RNA production exceeds 30µg, a second elution is recommended.

Protocol 2 Bead Grinding Method

This protocol adopts the bead grinding method, which is suitable for extracting high-yield RNA from various Gram negative or positive bacteria, as well as bacteria difficult to lyse.

1. Take 0.5-1.8ml bacterial culture medium (<1x10^9) in the exponential phase and transfer to a 2.0ml centrifuge tube. Centrifuge at 12,000 × g for 1 minute to collect bacteria. Discard the culture medium and invert it onto an absorbent paper to absorb the remaining liquid.
2. Add 300μl of Buffer STL/2-mercaptoethanol and a spoonful of glass beads (0.1-0.6mm) to the precipitate, vortex at maximum speed for 10 minutes, or transfer to a bead grinder for efficient bead grinding.

- **Vortex meter:** It is recommended to use MagMix A or efficient horizontal vortex meter MagMix B. These two products equipped with 2ml centrifuge tubes, can efficiently process 10-20 samples at once. The commonly used vortex instrument or constant temperature metal bath with oscillation function in the laboratory can also be used. When there is a constant temperature metal bath, a 2ml centrifuge tube is needed to prevent ineffective rotation of the tubes and reduce the grinding effect of the beads.
- **PowerLyzer bead grinder**: It is recommended to grind at 2,000rpm for 30 seconds, pause for 30 seconds, and then grind at 2,000rpm for another 30 seconds.
- **FastPrep24 bead grinder**: It is recommended to grind at 5m/s for 30 seconds, pause for 30 seconds, and then grind at 5m/s for 30 seconds.
- **Tissue Lysis II bead grinder**: It is recommended to grind at 25Hz for 5 minutes, readjust the position, and then grind at 25Hz for another 5 minutes.

3. **Brief centrifuge, add 500μl Buffer RLC/2-mercaptoethanol, vortex for 5~10 seconds, centrifuge at 12,000 × g for 1 minute.** Follow the step 4-11 of Protocol 1.