# RNApure Bacteria Kit (DNase I)

**Project number: R669890**

Storage conditions: -20° C.

## Products

| | |
|---|---|
| individual parts making up a compound | 50T |
| DNase I | 1000U |
| 10×Reaction Buffer | 1000μl |
| Buffer RL | 35ml |
| Buffer RW1 | 40ml |
| Buffer RW2 (concentrate) | 11ml |
| RNase- Free Water | 10ml |
| Spin Columns FL with Collection Tubes | 50 |
| with Collection Tubes with Collection Tubes | 50 |
| RNase-Free Centrifuge Tubes (1.5 ml) | 100 |

## Products

This kit adopts centrifugal adsorption columns with high efficiency and specific binding of nucleic acids and unique buffer system, which can rapidly extract total RNA from bacteria or cultured animal cells.The reaction can be completed in 30-40 minutes, and the extracted total RNA is extremely pure and free of protein and other contaminants, which is suitable for RT-PCR, Real-Time RT-PCR, microarray analysis, in vitro translation and other experiments.

Self-contained reagents: Lysozyme, β-mercaptoethanol, anhydrous ethanol (freshly opened or for RNA extraction).

## Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:

1) Use RNase-free plastics and tips to avoid cross-contamination.

2) RNase-free water should be used to prepare the solution.

(3) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.

2. Add β-mercaptoethanol to Buffer RL before use to reach a final concentration of 1%, e.g., add 10 μl of β-mercaptoethanol to 1 ml of Buffer RL. Buffer RL with β-mercaptoethanol can be stored at 4℃ for 1 month, if precipitation occurs, please heat to dissolve and use.
3. Anhydrous ethanol should be added to Buffer RW2 before first use according to the instructions on the reagent bottle label.
4. All centrifugation steps are carried out at room temperature if not otherwise specified, and all steps should be performed quickly.

## Procedure

1. Centrifuge at 12,000 rpm (~13,400 x g) at 4° C for 2 minutes to collect the organisms (maximum volume of organisms should not exceed 1 x 109) and carefully remove all supernatants.
Note: Supernatants that leave residues can interfere with the subsequent digestion process.
2. Thoroughly resuspend the organisms with 100 μl of TE buffer containing Lysozyme and incubate at room temperature. The specific formulation and incubation time are as follows:

| / | Final concentration of Lysozyme in TE buffer | incubation time |
|---|---|---|
| G-bacteria | 400μg/ml | 3-5 minutes |
| G+ Bacteria | 3mg/ml | 5-10 minutes |

3. Add 350 μl of Buffer RL (check that β-mercaptoethanol has been added before use), vortex and shake to mix (insoluble precipitate may appear in this step), add all of the solution and the precipitate to the filter columns (Spin Columns FL) that have been loaded into the collection tubes, and centrifuge at 12,000 rpm for 2 minutes.
4. Add 250 μl of anhydrous ethanol to the filtrate obtained in the previous step and mix well (a precipitate may appear at this point). Transfer the resulting solution together with the precipitate to a Spin Columns RM packed in a collection tube, centrifuge at 12,000 rpm for 1 min, discard the waste solution and put the column back into the collection tube.
5. Add 350 μl Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid and put the adsorbent column back into the collection tube.
6. Preparation of DNase I mixture: Take 52μl of RNase-Free Water, add 8μl of 10×Reaction Buffer and 20μl of DNase I (1U/μl) to it, mix well, and make a final volume of 80μl of reaction solution.
7. Add 80 μl of DNase I mixture directly to the adsorption column and incubate at 20-30° C for 15 minutes.

8. Add 350 μl Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid and put the adsorbent column back into the collection tube.
9. Add 500 μl of Buffer RW2 to the column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 1 min, and discard the waste solution.
10. Repeat step 9.
11. Place the adsorbent column back into the collection tube and centrifuge at 12,000 rpm for 2 minutes.
Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (zymography, PCR, etc.).
12. Load the adsorption column into a new RNase-Free collection tube, add 30-50 μl of RNase-Free Water to the middle of the adsorption membrane, leave it at room temperature for 1 minute, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -70° C to prevent degradation.
Note: 1) The volume of RNase-Free Water should not be less than 30 μl, too small volume affects the recovery rate.
2) If you want to increase the RNA yield, repeat step 12 with 30-50 μl of fresh RNase-Free Water.
If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 12 repeated.