(DP430) RNAprep Pure Cell/Bacteria Kit ——Bacteria

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Experiment Preparation

1. Bacteria culture, lysozyme (RT401, self-prepared. For bacterial total RNA extraction)
2. Ethanol, β-mercaptoethanol
3. Disposable sterile syringe (for DNase I preparation); Pipette and matched sterile RNase-Free tips (200 μl ,1 ml); 1.5 ml and 2.0 ml centrifuge tubes (RNase-free)
4. Fume hood; Grinder; Vortex oscillator; Refrigerated centrifuge

Experiment Preparation - Kit Preparation 1

Please add 96-100% ethanol in Buffer RW before use according to the volume indicated on the label of the bottle, and label the tube.

Preparation of DNase I storage solution

Dissolve the DNase I powder (1500 U) in 550 μl RNase-Free ddH_2O, gently mix well, and store in -20℃ (for up to 9 months) after aliquoting.

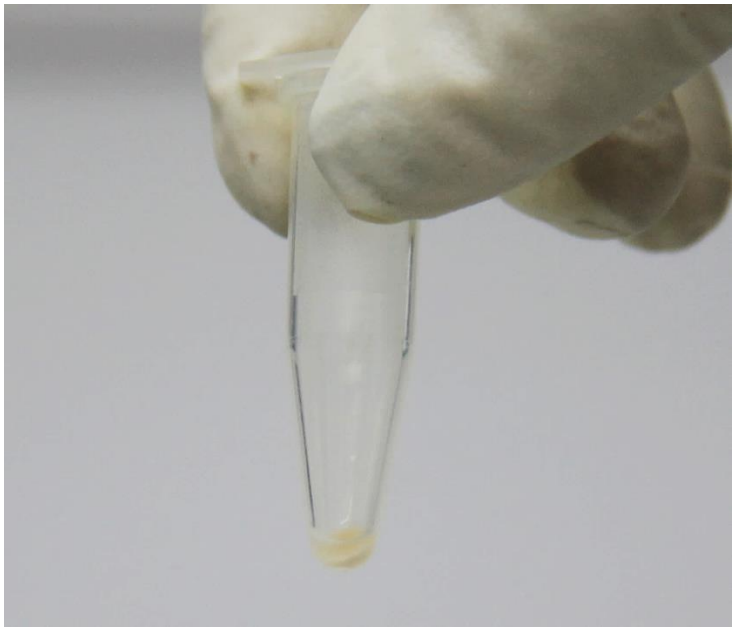
Note: The thawed the DNase I storage solution from -20℃ can be stored at 4 ℃ (for up to 6 weeks). Do not freeze again。

Experiment Preparation - Kit Preparation 2

It is suggested to operate this step in the fume hood

Add β-mercaptoethanol in Buffer RL to make 1% final concentration before operating. For example, add 10 μl β-mercaptoethanol to 1 ml Buffer RL. It is suggested to prepare the lysis buffer right before use. The prepared Buffer RL can be placed in 4℃ for one month. Buffer RL may precipitate during storage. If precipitation occurs, please heat and dissolve before use.

Step 1



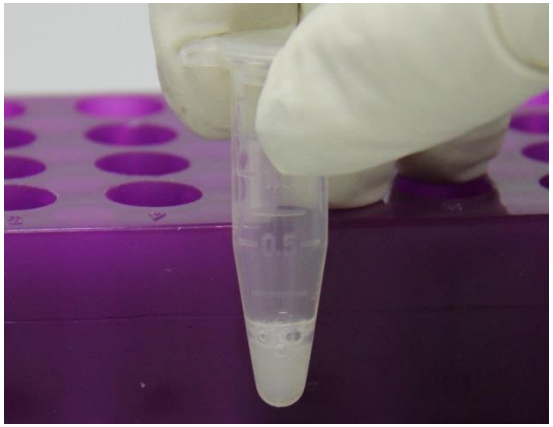
Centrifuge at 12,000 rpm (~13,400 g) at 4℃ for 2 min to collect bacteria (the maximum number of bacteria collected should not exceed 1×10^9). Carefully remove the supernatant of all the medium. All the following centrifugal steps are performed at room temperature (20-25℃).

Note: If the supernatant of medium is not removed completely, this will inhibit the cell wall digestion in step 2.

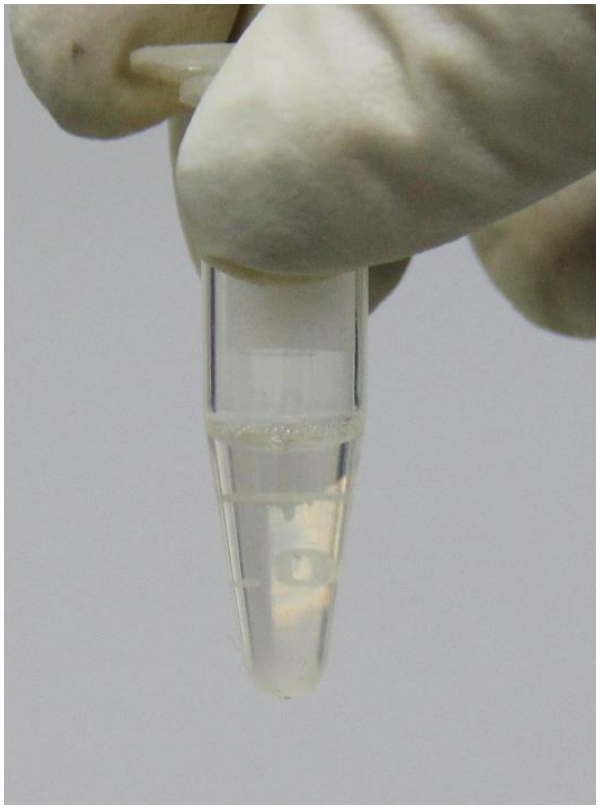
Step 2

Resuspend the bacteria pellet with100 μl Buffer TE containing lysozyme (self-prepared. Refer to the following table for the preparation method and the incubation time).

	Final concentration of lysozyme in Buffer TE	Incubation time (room temperature)
G^- bacteria	400 μg/ ml	3-5 min
G^+ bacteria	3 mg/ ml	5-10 min



Step 3



Add 350 μl Buffer RL (please add β-mercaptoethanol before use), mix thoroughly by vortex. If insoluble precipitation occurs, centrifuge at 12,000 rpm (~13,400×g) for 2 min, and transfer the supernatant to another centrifuge tube.

Step 4

Add 250 μl ethanol (96-100%) and mix thoroughly (precipitation may occur at this point).

Transfer the solution and precipitate to Spin Column CR3. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec, discard the waste liquid and place the Spin Column CR3 into the collection tube.

Step 5

Add 350 μl Buffer RW1 to Spin Column CR3. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec, discard the waste liquid and place the Spin Column CR3 into the collection tube.

Step 6

Preparation of DNase I working solution:

For 1 sample: Add 10 μl of DNase I storage solution into a new RNase-free centrifuge tube, then add 70 μl Buffer RDD and mix well gently (gently mix with a pipette).

For multiple samples extracted at the same time, please prepare the DNase I working solution together. It is recommended to prepare extra solution to avoid the situation that the total amount is insufficient due to the error of the pipette or the liquid attaching to the tips.

Step 7

Add 80 μl DNase I working solution to the center of Spin Column CR3, and place at room temperature for 15 min.

Step 8

Add 350 µl Buffer RW1 in Spin Column CR3. Centrifuge at 12,000 rpm (~13,400 g) for 30-60 sec, discard the waste liquid, and put the Spin Column CR3 back into the collection tube.

Step 9

Add 500 μl Buffer RW to the Spin Column CR3 (**make sure 96-100% ethanol has been added before use**), place at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec. Pour out the waste liquid and put the Spin Column CR3 back into the collection tube.

Step 10 Repeat step 9.

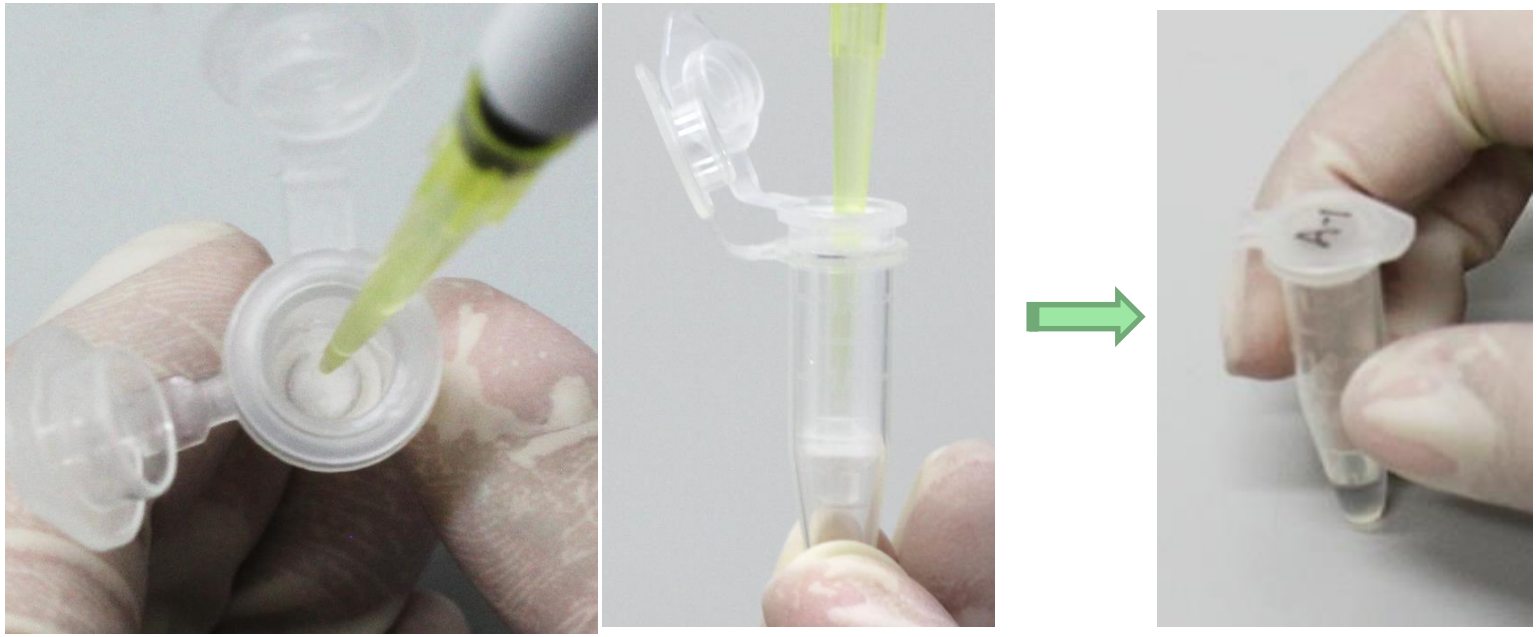
Step 11

Place the Spin Column CR3 in a new 2 ml collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 2 min to remove the residual liquid.

Place the Spin Column CR3 at room temperature for a moment, or place on the clean bench to ventilate for a moment to fully dry the membrane.

Note: Ethanol residues in Buffer RW can inhibit subsequent enzymatic reactions (RT, qPCR, etc.) experiments. However, avoid over-drying, or it will lead to RNA degradation or hard dissolution.

Step 12



Transfer the Spin Column CR3 into the centrifuge tube provided by the kit, add 30–100 μl RNase-Free ddH_2O, place at room temperature for 2 minutes and centrifuge at 12,000 rpm (~13,400×g) for 2 min.

The volume of elution buffer should not be less than 30 μl since too small volume affects the recovery efficiency. RNA should be kept at -70℃ to prevent degradation.