

TIANamp Bacteria DNA Kit

Kit Contents

Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
Proteinase K	1 ml
Spin Columns CB3	50
Collection Tubes 2 ml	50
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Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Pipet 1-5 ml bacterial culture suspension in a centrifuge tube, centrifuging for 1 min at 10,000 rpm (~11,500 × g), discard supernatant as possible.
2. Add 200 µl Buffer GA. Mix thoroughly by vortex.
3. Add 20 µl Proteinase K. Mix thoroughly by vortex.
4. Add 220 µl Buffer GB to the sample, vortex for 15 s, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
5. Add 220 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
6. Pipet the mixture from step 5 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl Buffer GD (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
8. Add 600 µl Buffer PW (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
9. Repeat Step 8.
10. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.
11. Place the Spin Column CB3 in a new clean 1.5 ml centrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).