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From Gram +/- Bacteria

Research Use Only | Store at Room Temp.

Product Code: 10027

Size: 100 preparations

Description

AddPrep Bacterial Genomic DNA Extraction Kit offer simple, rapid and cost-effective method for isolating genomic DNA from Gram Positive and Negative Bacteria, including Escherichia coli, Bacillus cereus and Staphylococcus aureus. The yield of genomic DNA extracted from 2.0 X 10^9 of gram-positive and negative bacteria (between 1.0 ~ 2.0 ml of overnight culture) is 5 ~ 15 μg. The genomic DNA extraction is based on spin column method with special buffers and without any solvents for a genomic DNA extraction. The extracted genomic DNA can be adjusted in variable applications, such as molecular biology experiments including PCR, blotting and so on.

Kit Components

Solution & Material	Size	Remarks
Spin column	100 ea	with collection tube
Lysis	25 ml	
Binding	25 ml	
Washing 1	30 ml	**Add Ethanol 22.5 ml before use**
Washing 2	12 ml	**Add Ethanol 48 ml before use**
Elution	25 ml	
Lysozyme Buffer	55 ml	
Lysozyme Sol.	1.2 ml X 2 tubes	50 mg/ml
Proteinase K Sol.	1.2 ml X 2 tubes	20 mg/ml

Storage and Stability

AddPrep Bacterial Genomic DNA Extraction Kit is stable for 3 years when stored in a constant temperature 15 ~ 35℃.

Before You Begin

1. Add ethanol to Washing 1 and Washing 2 Solution before use.
2. Check Lysis, Binding and Washing 1 Solution for any precipitation, and any precipitant can be dissolved by warming at 50℃.

1-A.Lysis protocol for Gram-negative bacteria

1-A-1. Harvest the overnight cultured cell 1 ml ~ 2ml by centrifuge at 13,000 rpm for 30 sec. with 1.5 ml tube (not provided).
1-A-2. Discard the supernatant.
1-A-3 Add 200 µl of Lysis Solution and 20 µl Proteinase K Solution (20 mg/ml) and resuspend the cell pellet by pipetting or vortexing.
1-A-4. Incubate it into 56℃ water bath for 10 minutes. Vortex occasionally during incubation to disperse the sample.
Optional RNase A treatment: If RNA-free genomic DNA is required, add the 20 µl of RNase A Solution (10 mg/ml, Not provided).
1-A-5. Add 200 µl of Binding Solution and 200 µl of absolute ethanol and mix well by pulse-vortexing for 15 sec.
1-A-6. Centrifuge at 13,000 rpm for 3 minutes.
1-A-7. Carefully transfer 500 ~ 600 µl of supernatant without pelletinto the upper reservoir of the spin column with 2.0 ml collection tube without wetting the rim.→ continue with step 2.

Note: If use the procedure of Gram-positive bacterial genomic DNA extraction, the yield of purified DNA of Gram-negative bacteria will be more 1.5~2.0 foldincreased than lysis protocol of Gram-negative bacteria.

1-B. Lysis protocol for Gram-positive bacteria

1-B-1. Harvest the overnight cultured cell 1 ml ~ 2ml by centrifuge at 13,000 rpm for 30 sec. with 1.5 ml tube (not provided).
1-B-2. Discard the supernatant.
1-B-3. Add 500 µl of Lysozyme Buffer and 20 µl of Lysozyme (50 mg/ml) and resuspend the cell pellet by pipetting or vortexing.
1-B-4. Incubate it into 37℃ water bath for 60 minutes.
Mix welloccasionally during incubation to disperse the sample
1-B-5. Centrifuge at 13,000 rpm for 3 minutes and discard the supernatant.
1-B-6. Add 200 µl of Lysis Solution and 20 µl Proteinase K Solution (20 mg/ml) and resuspend the cell pellet by pipetting or vortexing.
1-B-7. Incubate it into 56℃ water bath for 10 minutes. Vortex occasionally during incubation to disperse the sample.
Optional RNase A treatment: If RNA-free genomic DNA is required, add the 20 µl of RNase A Solution (10 mg/ml, Not provided).
1-B-8. Add 200 µl of Binding Solution and 200 µl of absolute ethanol and mix well by pulse-vortexing for 15 sec.
1-B-9. Centrifuge at 13,000 rpm for 3 minutes.
1-B-10. Carefully transfer 500 ~ 600 µl of supernatant without pelletinto the upper reservoir of the spin column with 2.0 ml collection tube without wetting the rim. → continue with step 2.

2. Centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

3. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

4. Add 500 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

5. Dry the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.

6. Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).

7. Add 100 ~ 200 µl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 min.

8. Elute the genomic DNA by centrifugation at 13,000 rpm for 1 min.