Gram-positive Bacterium Genomic DNA Extraction Kit

Cat No.: EXD0212

Size: 50T/ 100T

Storage: Store in dry and temperate condition(15℃-25℃), re-test period for 12 months. Storage at 2℃-8℃for longer. Enzymes will be shipped as accessories, please store at -20℃ after receiving them.

Kit Contents:

Component	50T	100T
Lysozyme	0.3g	0.5g
RNase A	1ml	1ml×2
Proteinase K	1ml	1ml×2
Solution A	10ml	20ml
Solution B	10ml	20ml
Washing buffer	15ml	15ml×2
Elution buffer	15ml	30ml
Adsorption column	50 units	100 units
Collection tube	50 units	100 units

Product Description

Gram-positive Bacterium Genomic DNA Extraction Kit is based on a centrifugal adsorption column that specifically binds DNA and a unique buffer system to extract Gram-positive genomic DNA. The spin column is made of new type of silica membrane which can bind DNA specifically, and can remove impurity proteins and other organic compounds in cells to the utmost extent. DNA purified by Gram-positive Bacterium Genomic DNA Extraction Kit is highly suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Protocol

Note: Please add Absolute Ethanol to the Washing Buffer before use. Please refer to the label on the bottle for the added volume. Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

1. Take 1ml of bacterial cultures by centrifugation for 1min at 12,000rpm in a microcentrifuge, remove the supernatant as much as possible.
2. Add 200µl, 20mg/ml Lysozyme(add buffer 20mM Tris, PH 8.0, 2mM Na_2-EDTA, 1.2% Triton X-100 to Lysozyme powder, keep in 37 ℃ for 30 min at least). Add 20µl RNase A(100mg/ml) if remove RNA.
3. Completely resuspend the bacterial pellet in 200µl of Solution A, incubate at room temperature

for 30-60min.

4. Add 20µl Proteinase K(10mg/ml), mix thoroughly, incubate at 55℃for 30-60min. Invert the tube several times during incubating. The mixture should be clear.
5. Add 200µl Solution B, mix thoroughly. White precipitates may form, incubate at 75℃for15-30 min， white precipitates will disappear. It shows that the sample does not digested thoroughly if the solution not clear, which may make the amount and purity of DNA extraction lower and block the column.
6. Add 200µl Absolute Ethanol to the sample, and mix thoroughly. Flocculent precipitate may also form on addition of ethanol. Add the mixture into a adsorption column, incubate at RT for 2 min.
7. Centrifuge at 12,000 rpm for 2min. Discard the flow-through and re-use the collection tube in the next step.
8. Wash the Adsorption Column with 600µl Washing buffer, centrifuge at 12,000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.

   Note: Washing buffer must be diluted with absolute ethanol before use.
9. Repeat step 8 with another 600µl Washing buffer.
10. Centrifuge the empty column at 12,000rpm for 2min. Incubate at room temperature or 50℃ to dry the column. It is critical for removing ethanol from the column.
11. Place column into a new clean centrifuge tube. Add 50-200µl Elution buffer(pre-heated to 65℃) onto the column matrix, incubate at room temperature for 5min. Centrifuge at 12,000rpm for 1 min.
12. To increased DNA concentration, add the solution obtained from step 11 to the center of membrane again. Incubate at room temperature (15-25℃) for 2 min, and then centrifuge for 2 min at 12,000 rpm.

Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size and amount.
2. If the precipitation appears, dissolve at 65℃ water bath please, which will not affect on using.
3. If Adsorption Column is blocked , the time of centrifugation can be extended.
4. If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0, below 7.0 will affect elution efficiency. DNA product should be stored at -20 。C to avoid degradation.
5. Detect the concentration and purity of DNA: The purity of plasmid DNA influenced by many factors, the DNA purity can be detected by Agarose gel electrophoresis and Ultraviolet spectrophotometer. DNA should have absorption peak in OD_{260}, OD_{260}=1 is equal to 50µl double-stranded DNA, 40µl single-stranded DNA. OD_{260}/OD_{280} should be 1.7-1.9, the value will be lower if using distilled water in eluting, but do not show the purity is low.