Genomic DNA extraction kit for Gram-positive bacteria

Cat No.: D1650

Package: 50T/ 100T

Storage: Keep it at room temperature, and the reinspection period is one year. (Note: RNase A, lysozyme, proteinase K are shipped as accessories and stored at-20℃)

Kit content:

Component	D1650-50T	D1650-100T	Storage
Lysozyme	3mL	3mL×2	-20℃
RNase A	100μL	100μL×2	-20℃
Proteinase K	1mL	1mL×2	-20℃
Solution A	10mL	20mL	RT
Solution B	10mL	20mL	RT
Wash Buffer	15mL	15mL×2	RT
Elution Buffer	15mL	30mL	RT
Column	50 个	100 个	RT
Collection Tube	50 个	100 个	RT
Instruction	1 份	1 份	-

Product Description:

This kit uses a centrifugal adsorption column that can specifically bind DNA and a unique buffer system to extract genomic DNA from Gram-positive bacteria. The silicon matrix material used in the centrifugal adsorption column is the unique new material of the company, which can be highly efficient and specific DNA adsorption and maximize the removal of impurity proteins and other organic compounds in cells. The extracted genomic DNA fragments are large, of high purity and of stable and reliable quality. The genomic DNA extracted using this kit can be used for a variety of routine operations, including enzyme digestion, PCR, library construction, Southern hybridization, and other experiments.

Operation steps (for reference only):

Before use, please add absolute ethanol to the rinse solution, and refer to the label on the bottle body (45mL of absolute ethanol should be added to each bottle separately). All centrifugation steps were performed at room temperature using a benchtop centrifuge.

1. 1 mL of bacterial medium was removed by centrifugation at 12000rpm for 1min, and the supernatant was aspirated as far as possible.
2. 200μL of solution A was added to the body, shaken or blown with a pipete to fully suspend the body, then 50μL of lysozyme was added and treated at 37℃ for more than 30min. (If RNA removal is required, 2μL RNase A of solution can be added).
3. 20μL of proteinase K was added to the tube, fully mixed, and digested at 55℃ for 30-60min.

During digestion, the centrifuge tube could be mixed several times until the sample was completely digested. At this time, the bacterial liquid was bright and thick.

4. Add 200μL of solution B to the tube, and mix well. If white precipitation occurs, it can be placed at 75℃ for 15-30min, and the precipitation will disappear without affecting the subsequent experiments. If the solution is not clear, it means that the sample is not digested thoroughly, which may cause the amount of DNA extraction and purity reduction, and may block the adsorption column.
5. Add 200μL of anhydrous ethanol to the tube and mix well. At this time, flocculent precipitation may occur, which does not affect the extraction of DNA. The solution and flocculent precipitation can be added to the adsorption column and stand for 2min.
6. Disige at 12000rpm for 2min and put the adsorption column into the collecting tube.
7. Add 600μL of rinse to the adsorption column (check whether absolute ethanol has been added before use). After centrifugation at 12,000rpm for 1min, the waste liquid was discarded and the adsorption column was placed into the collecting tube.
8. Add 600μL of rinse solution to the adsorption column, centrifuge at 12000rpm for 1min, discard the waste solution, and put the adsorption column into the collecting tube.
9. Centrifuge at 12000rpm for 2min and leave the adsorption column open at room temperature or 50℃ temperature box for several minutes to remove the residual rinse solution from the adsorption column, otherwise the ethanol in the rinse would affect subsequent experiments such as enzyme digestion, PCR, etc.
10. The adsorption column was placed in a clean centrifuge tube, and 50-200μL of eluent preheated with 65℃ water bath was dropped to the center of the adsorption membrane, placed at room temperature for 5min and centrifuged at 12000rpm for 1min.
11. The eluate from centrifugation was added to the adsorption column and centrifuged at 12000rpm for 2min to obtain high quality bacterial genomic DNA.

Note:

1. Samples should avoid repeated freezing and thawing, otherwise it will cause small extracted DNA fragments and decreased extraction amount.
2. If the solution in the kit is precipitated, it can be redissolved in a 65℃ water bath before use, without affecting the extraction effect.
3. If the centrifugation step in the experiment is blocked, the centrifugation time can be extended appropriately.
4. The volume of elution buffer should not be less than 50μL, the small volume will affect the recovery efficiency; the pH value of eluate also affects the elution efficiency, the pH value should be about 8.0 (the pH can be adjusted to this range), and the pH value lower than 7.0 will reduce the elution efficiency. The DNA product should be stored at-20℃ to prevent DNA degradation.

5. DNA concentration and purity detection: the size of the resulting genomic DNA fragments is related to the storage time of the sample, the shear force during the operation and other factors. The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. The DNA should have a significant absorption peak at OD260, with an OD260 value of 1.0 equivalent to approximately 50μg/mL double-stranded DNA, 40μg/mL single-stranded DNA. The OD260/ 0D280 ratio should be 1.7-1.9. If the elution buffer is not used, while the deionized water is used, the ratio will be low, because the pH and the presence of ions will affect the light absorption value, but it does not indicate low purity.

Related products:

D1010	*6×DNA Loading Buffer*
T1060	*50×TAE Buffer*
T1050	*5×TBE Buffer*
ML060	*D2000 DNA Ladder*
ML400	*1kb DNA Ladder*
G8142	*Gold View IInucleic acid stain (5000×)*
D1100	*Plasmid small-amount extraction kit*