For research use only

Version Number：2.0-1909

Bacterial DNA Isolation Kit

For purification of total DNA from bacteria using≤3ml culture medium (maximum 2×10^9 cells)

Kit content	DE-05311	DE-05312	DE-05313
	50 T	100 T	250 T
Buffer ML1	25ml	50ml	125ml
Buffer ML2	25ml	50ml	125ml
Buffer PW	30ml	60ml	150ml
Buffer WB	25ml	50ml	125ml
Buffer EB	10ml	20ml	50ml
Foregene Protease	1.25ml	1.25ml x2	6.25ml
Lysozyme	250mg	500mg	500mg x2
Buffer TE	5ml	8ml	10ml
DNA-Only Column	50 sets	100 sets	250 sets
IFU	1 piece	1 piece	1 piece

*： Buffer ML2 and Buffer PW contain irritating desalted salt. Please wear gloves and take relevant protective measures when operating.

Product introduction

This kit provides a quick and easy method of extracting genomic DNA from cultures of bacteria (Gram-negative and Gram-positive) from various sources; it can process less than 3ml of bacterial cultures in the logarithmic growth phase (1×10^9 bacteria). The kit is matched with high-efficiency Foregene Protease, which allows 15-50μg of high-quality genomic DNA to be extracted within 1 hour. In addition, the kit can also extract genetic material other than the genome, such as plasmids, Cosmid, BAC, etc.

The DNA-only silica gel matrix material used in the spin column is a unique new material of the company, which can efficiently and specifically adsorb DNA. The maximum adsorption capacity for DNA is 80μg. The unique buffer and elution system can maximize the removal of RNA, Impurity proteins, ions and other organic compounds in cells. The extracted genomic DNA fragment is large, high purity, stable and reliable quality, and the size of the DNA fragment is stable at about 23kb.

Product Information

Format	Spin column	Purification component	Foregene column, reagent
Flux	1-24 samples	Time per prep	~60 min (24 samples)
Centrifuge	Desk centrifuge	Plant lysate separation	Centrifugal separation
DNA Purification column loading	80μg	Liquid content of spin column	800μl
Elution volume	100-200 μL	Bacteria processing volume	≤3ml(Cultivate bacteria for 16-20hr)

Storage Condition

The kit can be stored for 24 months under dry conditions at room temperature (15–25℃). If you need to store longer time can be placed in 2-8℃. Foregene Protease solution has a unique formula, stored at room temperature for a long time (3 months) with activity; If stored at 4℃, its activity and stability will be better, so it is recommended to store at 4℃, remember not to store at-20℃.

Note: Solution is prone to precipitate if stored at low temperature. Before use, be sure to place the solution in the kit at room temperature for a period of time, if necessary, can be preheated in 37℃ water bath for 10 minutes, to dissolve precipitation, mixed before use.

DNA yield and purity

The amount of genomic DNA obtained by using the Bacterial DNA isolation kit to process bacteria is related to the source of the sample, storage time, culture time and other factors. Generally, the bacterial genomic DNA obtained under normal circumstances is the sum of the DNA in the bacterial cell, and the genomic DNA yield of the bacteria cultured overnight in 3ml is about 15-50μg. In actual operation, the amount obtained may differ slightly from the data.

Note: (Be sure to read the notes carefully before using)

◆ The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the yield will decrease.

◆ One purification column of this kit can process up to 3ml of overnight cultured bacteria; if the amount of bacteria is greater than 3ml, please divide it into multiple purification columns for processing, and finally pool the eluted genomic DNA together.

◆ Before using the kit, be sure to check whether the WB has been added with absolute ethanol according to the instructions. Add 60ml of absolute ethanol (DE-05311), 120ml of absolute ethanol (DE-05312), and 300ml of absolute ethanol (DE-05313) to Buffer WB before use.

◆ Before use, carefully check whether there is any precipitation in Buffer ML1, Buffer ML2 and Buffer PW. If there is precipitation, please dissolve it at 37°C and mix it before use.

◆ Elution volume: Buffer EB should not be less than 100μl, otherwise it will affect the DNA yield.

◆ Remember not to add RNase to any buffer.

◆ All centrifugation steps are centrifugation at room temperature (15-25℃) in a benchtop centrifuge.

◆ All experimental steps are carried out at room temperature (15-25℃).

Operating Steps

Before use, please add absolute ethanol to Buffer WB. Please refer to the label on the bottle for the added volume.

1. Take≤3ml(1×10^9) of overnight cultured bacterial solution, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min at room temperature, and absorb the supernatant as much as possible. Due to different sources of bacteria, please follow steps 1a and 1b below.

1a.Gram-negative bacteria: collect the bacteria, centrifuge to remove the supernatant, and proceed directly to step 2.

1b.Gram-positive bacteria: collect the bacteria, remove the supernatant by centrifugation, add **160μl** Buffer EB, and resuspend the bacteria; Add **40μl** Lysozyme (100mg/ml), vortex and mix, and place at 37℃ for 30min;7,200rpm(~5,000×g) centrifuge for 3 min, discard the supernatant and proceed to step 2.

2. Add **380μl** Buffer ML1 to the pellet, and vortex until the cells are completely suspended.
3. Add 20μl Foregene Protease and vortex to mix.
4. Place the centrifuge tube in a metal bath at 65℃ for 20-30min, and vortex every 10min to mix.
5. Add **380μl** Buffer ML2 to the centrifuge tube, invert and mix, and place in a metal bath at 65℃ for 10 minutes.
6. Centrifuge at 12,000 rpm (~13,400×g) for 5-10 minutes.
7. Pipette **750μl** of supernatant into a new 2ml centrifuge tube.

Note: When sucking the supernatant, avoid the sediment from being sucked in. If there are many solid impurities in the sucked supernatant, Step 6 can be repeated once.

8. Add **150μl** of ethanol (96-100%), shake vigorously until well mixed, and a flocculent precipitate may appear.
9. Transfer the mixture and the flocculent pellet to a spin column (DNA-Only Column),centrifuge at 12,000rpm (~13,400 ×g) for 1 min and discard the waste liquid in the collection tube.
10. Put the spin column back into the collection tube, add **500μl** Buffer PW to the spin column, centrifuge at 12,000rpm (~13,400 ×g) for 1 min and discard the waste liquid in the collection tube.
11. Put the spin column back into the collection tube, add **700μl** Buffer WB to the spin column, centrifuge at 12,000rpm (~13,400 ×g) for 1 min and discard the waste liquid in the collection tube.
12. Repeat step 11 one more time.
13. Put the spin column back into the collection tube and centrifuge the empty tube at 12,000 rpm (~13,400×g) for 2 min.
14. Transfer the spin column to a new 1.5ml centrifuge tube, drop **100μl** preheated Buffer EB at 65℃ (do not add the eluent to the pressure ring, otherwise a larger volume of eluent will be lost) into the center of the membrane.Leave at room temperature for 5 min, centrifuge at 12,000 rpm (~13,400×g) for 1 min. Drop **100μl** pre-warmed Buffer EB to the center of the membrane again, which has been heated at 65℃, centrifuge at 12,000 rpm (~13,400×g) for 1 min.Combine the eluates from the two collections

Note: If you want to increase the concentration of DNA, you can add the solution from the first centrifugation back to the spin column,centrifuge at 12,000 rpm (~13,400×g) for 1 min.