

## Bacterial Genomic DNA Extraction Kit

**Cat No.:** EXD0205

**Package:** 50T/ 100T

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

### Kit Contents:

| Component         | 50T      | 100T      |
|-------------------|----------|-----------|
| 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    | 10ml     | 20ml      |
| Adsorption column | 50 units | 100 units |
| Collection tube   | 50 units | 100 units |
| Instructions      | 1 unit   | 1 unit    |

### Product Description

Bacterial Genomic DNA Extraction Kit is based on silica membrane technology and provides special buffer system for many kinds of sample's DNA extraction. The spin column is made of new type of silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications. DNA purified by Bacterial 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. Pellet 1ml of bacterial cultures by centrifugation for 1min at 12,000rpm in a microcentrifuge. Remove the supernatant as much as possible.
2. Completely resuspend the bacterial pellet in 200µl of Solution A, add 20µl RNase A, mix thoroughly and incubate at RT for 15-30min.

Note: For Gram-positive Bacterium, add 200µl Lysozyme (20mg/ml) for 30min at 37 °C before the step 2. Buffer prepare: 20 mM Tris, pH 8.0; 2 mM Na<sub>2</sub>-EDTA; 1.2% Triton X-100.

3. Add 20 $\mu$ l Proteinase K(10mg/ml), mix thoroughly, incubate at 55°C for 30-60min. Invert the tube several times during incubating. The mixture should be clear.
4. Add 200 $\mu$ l Solution B, Mix thoroughly. White precipitates may form, incubate at 75°C for 15-30 min, White precipitates will disappear. It shows that the sample does not digest thoroughly if the solution not clear, which may make the amount and purity of DNA extraction lower and block the column.
5. Add 200 $\mu$ l ethanol (96-100%) to the sample, and mix thoroughly by vortex. A white precipitate may form on addition of ethanol. Add the mixture into a Adsorption column, incubate at RT for 2min.
6. Centrifuge for 2min at 12,000 rpm. Discard the flow-through and re-use the collection tube in the next step.
7. Wash the Adsorption Column with 600 $\mu$ l 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.
8. Repeat step 7 with another 600 $\mu$ l Washing buffer.
9. Centrifuge the empty column at 12,000rpm for 2min. Incubate at room temperature or 50°C to dry the column. It is critical for removing ethanol from the column.
10. Place column into a new clean centrifuge tube. Add 50-200 $\mu$ l Elution buffer (been heated to 65°C) onto the column matrix, incubate at room temperature for 5min. Centrifuge at 12,000rpm for 1 min.
11. For increased DNA concentration, add the solution obtained from step 10 to the center of membrane again. Incubate at room temperature (15-25°C) for 2-5 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.
2. If the precipitation appears, dissolve at 65°C water bath please, which had 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 50 $\mu$ L, 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<sub>260</sub>, OD<sub>260</sub>=1 is equal to 50 $\mu$ l double-stranded DNA, 40 $\mu$ l single-stranded DNA. OD<sub>260</sub>/OD<sub>280</sub> 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.

