Bacteria DNA Extraction Kit

The kit uses magnetic beads to adsorb DNA to achieve the goal of rapidly purifying bacterial genome DNA. Suitable for the extraction of genomic DNA with high purity from different bacteria in the sample, Using the genomic DNA Purification Kit (OD260-OD320) / (OD280-OD320) were in the range of 1.7 to 2, can be used for PCR/RT-PCR, Real-time PCR/Real-time RT-PCR, the two generation sequencing experiments in various molecular biology gene chips.

1. **Composition, storage and stability**

Items	**Amount**
Kit size	50 preps
lysateAX	15 ml
lysateB	1 tube
solutionA	1 ml
Magnetic bead A	500 μl
Washing liquid A	15 ml
Washing liquid S	18 ml
Eluent A	10 ml

Lysate AX**:** lysate, stored in airtight condition at room tempera

Magnetic bead A: Magnetic bead， stored in airtight condition at room tempera

Lysate B: Storage of freeze-dried protease K, please placed at -20℃; after using dissolved A dissolved, can be stored for 1 months at Solution A: The protease K solution is stored at 4 ℃.

Washing liquid A: add a specified amount of alcohol, mixed evenly, stored in airtight condition at room tempera

Washing liquid S：add a specified amount of alcohol, mixed evenly, stored in airtight condition at room tempera

Eluent A：Eluent No DNA/RNA enzyme activity ,stored in airtight condition at room tempera

2. Attention

A. low temperature storage of pyrolysis liquid AX may lead to white flocculent floating matter. It can be heated to white precipitate before being used in the thermostatic water tank, so that it can not be dissolved.

B. Magnetic bead A must be fully mixed before use.

C. In order to avoid cross contamination, the used reagents, pre assembled boards and mixing sleeves should be safely disposed of in time.

3. Preparation

A. Used for the first time, the lysate of B with 350 uL A solution fully dissolved.

Attention: If the long-term placement will have B cracking liquid solid precipitation, but does not affect the extraction effect, before each use to fully mix.

B. Prepare isopropanol and absolute ethanol (pure, sterile, no RNase pollution).

C. Magnetic rack.

D. Washing liquid A and washing liquid S, after opening, immediately follow the label marked amount to add the corresponding alcohol, and in the reagent bottle on the mark.

4. Operation

The following steps are taken to extract genomic DNA from a bacterial culture overnight for 1 ml:

A. Take 1 ml overnight culture of liquid to 1.5 ml EP tube, 12000 RPM centrifugation for 1 minutes, enrichment bacteria, as far as possible suction clean supernatant.

Attention: If the sample is gram negative bacteria and Staphylococcus aureus directly to the next step; if the samples were gram positive bacteria (Staphylococcus excepted) can be added to 25 l 20 mg/ml lysozyme (customer owned) repeated pipetting. Cell suspension fully, 37 ℃digestion 30 ~ 60 minutes.

B. With 300ul AX, 5uL lysate, repeated pipetting. Cell suspension can also be fully vortex oscillator.

C. The EP tube is placed in a constant temperature tank for 70 ℃ and 10~30 minutes until the white precipitate is dissolved completely.

D. Adding oscillation blending beads A 10u L, 350u l isopropanol (customer owned), the room temperature upside down mix for 5 minutes.

E. The EP tube is placed on the magnetic shelf, stationary to the magnetic beads, all adsorption to the pipe wall, suction tube fluid.

F. Remove the magnetic frame, adding 500U L washing liquid A, oscillating mixing for 1 minutes. The EP tube is placed on the magnetic frame, and the magnetic beads are fixed to the tube wall, and the liquid in the suction tube is sucked.

G. Remove the magnetic frame, adding 500U L washing liquid S, oscillating mixing for 1 minutes. The EP tube is placed on the magnetic frame, and the magnetic beads are fixed to the tube wall, and the liquid in the suction tube is sucked.

H. Repeat step 7 at once. Open the tube and let dry for 5 minutes.

J. Remove the magnetic frame, adding 100 ~ 200u L eluent A, flick EP tube beads all infiltration in the liquid, 65 ℃ water bath for 10 minutes, every 2-3 minutes mixing rocked EP tube.

K. The EP tube is placed on the magnetic shelf, and the magnetic beads are fixed to the tube wall, and the supernatant is absorbed into a new EP tube to obtain genomic DNA.

5. Commonly problem and suggestion

Commonly problem	reason	suggestion
Yield is low	The concentration of bacteria is too low	Increased cell entry before incubation or increased cell collection (2~5 ml cell centrifugation)
	The cultures were not in the early stage of logarithmic growth	The longer preserved bacteria can be used overnight for 2~3 times
	Incomplete lysis	Sample concentration is too high or too much sampling, it is recommended to extend the cracking time or reduce the amount of samples