

Bacterial (G +) Genomic DNA Kit (Magnetic Bead Method)

Cat No.: DM1170M

Package: 50T/ 100T

Storage: 2-8°C (Note: RNase A is stored in accessory form, -20°C; Proteinase K, lysozyme, magnetic beads, 4°C is stored, do not move cold)

Component	50T	100T	Storage
Lysate	10mL	20mL	RT
Binding Buffer	15mL	30mL	RT
Wash Buffer 1	25mL	50mL	RT
Wash Buffer 2	6.25mL	12.5mL	RT
Lysozyme	750mg	750mg×2	2-8°C
Proteinase K	20mg	20mg×2	2-8°C
Lysozyme Liquid	9mL	18mL	RT
RNase A	100μL	100μL×2	-20°C
Elution Buffer	5mL	10mL	RT
Magnetic Bead	1mL	1mL×2	2-8°C, 切勿冻存
Instruction	1 份	1 份	-

Product description:

Magnetic bead method bacterial genome DNA cassette, make bacteria in the lysate lysis completely, under the action of binding liquid to promote genomic DNA and magnetic beads specifically identify and efficient binding, after the rinse can maximize remove impurity protein and other organic compounds, under the action of external magnetic field force can separate DNA from the sample. The extracted genomic DNA with high concentration and excellent purity can be applied to various downstream experiments of molecular biology, suitable for Gram-positive bacteria.

Advantages:

The extraction of bacterial genomic DNA by magnetic bead method has incomparable advantages to the traditional column method. Compared with the column method, it greatly reduces the experimental time and extracts high-quality bacterial genomic DNA that can be applied to all kinds of molecular biology downstream experiments. It has the advantages of simple operation, short time, safe and non-toxic, can complete automatic extraction and so on.

Operation steps (for reference only):

Before use, add isopropanol/ absolute ethanol to the rinse solution, refer to the label on the reagent volume (50L/ 100T rinse 125mL/ 50mL, 50T/ 100T rinse 218.75mL/ 37.5mL absolute ethanol alone).

Lysozyme solution configuration: Add 7.5mL of lysing solution to 750mg lysozyme powder to 100mg/





mL of lysozyme solution and lysozyme solution-20°C.

Protease K solution configuration: add 1mL of lytic solution to 20mg protease K powder, mix 20mg/mL of proteinase K solution, and keep the good protease solution-20°C.

- 1、 1 mL of overnight culture solution was added to the EP tube and centrifuged at 12000rpm for 1 min to remove the supernatant as far as possible.
- 2、 500μL was resuspended with 70% ethanol in an ice bath for 20min at 12000rpm and centrifuged for 1min to remove the supernatant as far as possible.
- 3、 Add 200μL of buffer (20mM Tris, pH8.0; 2mM Na₂-EDTA; 1.2% TritonX-100) and 150μL of lysozyme solution for 1h, extend the incubation to 3h, and mix well every 20min.
- 4、 The sample was centrifuged at 12000rpm for 1 min and then the supernatant was aspirated as far as possible.
- 5、 Add 200μL of lysate to the bacteria, vortex and shake, or blow the straw body repeatedly with a pipette gun to fully suspend the bacteria. Add 20μL proteinase K, mix well, digest in a 65°C water bath for 30min, and mix well every 10min. The bacterial solution is clear and is completely digested.
- 6、 The EP tube was removed from the water bath, the temperature dropped to room temperature for 2μL RNase A, the suction gun was blown and mixed, and let at room temperature for 10min.
- 7、 Add 130μL of binding solution, blow and mix with a pipette gun, and bath for 10min in a 65°C water bath.
- 8、 The EP tube was removed from the water bath, and 20μL magnetic beads were added after dropping to room temperature, vortex and shaken at room temperature for 5 min. Place the EP tube in the magnetic frame for magnetic separation. After the magnetic beads are fully adsorbed to the magnetic frame, remove the liquid along the tube wall with a pipette gun. Be careful not to absorb the magnetic beads.
- 9、 Add 500μL of rinse solution 1 (25mL isopropyl alcohol before 50T and 50mL isopropyl alcohol before 100T) and mix with a vortex oscillator. Place the centrifuge tube in the magnetic frame. After the magnetic beads are completely adsorbed to the magnetic frame, remove the liquid along the pipe wall with a pipette. Be careful not to absorb the magnetic beads.
- 10、 Repeat step 9.
- 11、 Add 500μL of rinse solution 2 (check for absolute ethanol before use) and mix with a vortex oscillator. Place the centrifuge tube in the magnetic frame. After the magnetic beads are completely adsorbed to the magnetic frame, remove the liquid along the tube wall with a pipette. Be careful not to absorb the magnetic beads.
- 12、 Open the lid of the centrifuge tube and set it to dry at room temperature for 2min. Just observe the liquid volatilization of the wall and bottom and the smooth surface of the magnetic beads. Pay attention to the drying time can not be too long, which will make the magnetic beads not easy to be eluted.
- 13、 After 5 min in a 60°C water bath, 50-100μL is added to the above centrifuge tube, vortex and mix the centrifuge tube in the magnetic frame. After the magnetic beads are fully adsorbed to the magnetic frame, and the solution is sucked into the new centrifugal tube along the tube wall with a pipette gun. Be careful not to absorb the magnetic beads.
- 14、 The sample was centrifuged at 2000rpm for 1 min and the resulting solution was a purified genomic DNA sample and stored at -20°C.
- 15、

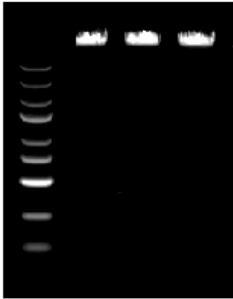
Note:

- 1、 The magnetic beads before use with a scroll oscillator.
- 2、 The magnetic beads were stored in a 4°C refrigerator.



- 3、 Do not freeze the magnetic beads and leave them in a dry state.
- 4、 The genome extracted using a fresh bacterial solution was of good quality.
- 5、 Lysozyme is best used in use.
- 6、 Samples should avoid repeated freezing and thawing, otherwise causing a decrease in extraction volume.

Experimental Result:



Legend:

1 mL overnight *S. aureus* genome extraction was eluted in a volume of 80 μ L at a concentration around 240ng/ μ L, loaded with 3 μ L by electrophoresis on 1% agarose gel and electrophoresis at 150V for 20min

M: D2000 plus DNA Ladder

Related products:

DM1700 Genome Extraction of Animal Tissues (Magnetic Bead Method)

DM1300 DNA Product Purification Kit (Magnetic Bead Method)

DM1200 DNA Agarose Gel Recovery Kit (Magnetic Bead Method)

DM1100 Plasmid Small Extraction Kit (Magnetic Bead Method)

DM1800 Whole Blood Genomic DNA Extraction Kit (Magnetic Bead Method)

DM1600 Bacterial Genomic DNA Extraction Kit (Magnetic Bead Method)



