



Bacterial DNA Extraction Kit

Intended Use

The SpiNXT Bacterial DNA Extraction kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of neither a disease nor it is suitable for administration to humans or animals. All due care and attention should be exercised in the handling of the products.

Intended User

The assay is intended to be performed by a laboratory professional in research laboratory.

Test Principle

Bacterial cells are lysed to release their DNA, using enzymes and detergents. Proteins are denatured and removed, allowing the DNA to be released. The released DNA binds to a solid phase, i.e., silica membrane, in the presence of salts. Impurities are removed through washing steps, leaving only the DNA bound to the solid phase. The purified DNA is then eluted from the solid phase using a low-salt buffer or water. SpiNXT Bacterial DNA Extraction kit efficiently and effectively extracts high-quality DNA from bacterial cells, suitable for downstream applications like PCR, sequencing, and genotyping.

Summary

SpiNXT Bacterial DNA Extraction kit is designed for the rapid preparation of genomic DNA from 2×10^9 viable bacterial cells. Purification is based on silica membrane based spin columns as the separation matrix. It allow isolation of gDNA from both gram negative and gram positive cultures. The kit combines the features and advantages of silica binding with a micro spin format, and eliminates the need of hazardous compounds such as phenol/chloroform extraction or alcohol precipitation and involves minimal handling.

The purified DNA is suitable for various down-streaming molecular biology applications such as PCR, Cloning, Next generation sequencing and so on.

Storage, Operating Conditions and Stability

- The kit has a shelf life of 18 months from the date of manufacturing.
- The test kit and its component are stable until the expiration date mentioned on the kit box.
- All the kit Components is shipped and stored at 15°C to 25°C except Lysozyme is shipped and stored at -20°C.
- RNaseA (10 mg/ml). is shipped at 15°C to 25°C and stored at 2°C to 8°C.

Reagents Provided

Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0642
Buffer DLB	G2MBR3-1370-1	1 X 25 ml
Buffer DLB-20	G2MBR3-1371-1	1 X 1.2 ml
Buffer W1	G2MBR3-1372-1	1 X 20 ml
Buffer W2	G2MBR3-1373-1	1 X 20 ml
Proteinase K	G2MBR3-1374-1	1 X 30 mg
Protease Dissolve Buffer	G2MBR3-1375-1	1 X 4 ml
Buffer EB	G2MBR3-1376-1	1 X 10 ml
Lysozyme	G2MBR3-1377-1	1 X 1 ml
RNaseA	G2MBR3-1378-1	1 X 250 µl

Consumables Provided

Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0642
Mini Column	1 X 50 Nos.
Collection Tubes	1 X 50 Nos.

Reagents Provided

Table 2a. (For 250 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0643
Buffer DLB	G2MBR3-1370-2	1 X 110 ml
Buffer DLB-20	G2MBR3-1371-2	1 X 6 ml
Buffer W1	G2MBR3-1372-2	1 X 90 ml
Buffer W2	G2MBR3-1373-2	2 X 45 ml
Proteinase K	G2MBR3-1374-2	1 X 120 mg
Protease Dissolve Buffer	G2MBR3-1375-2	1 X 10 ml
Buffer EB	G2MBR3-1376-2	1 X 30 ml
Lysozyme	G2MBR3-1377-2	1 X 5 ml
RNaseA	G2MBR3-1378-2	1 X 1.25 ml

Consumables Provided

Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0643
Mini Column	2 X 125 Nos.
Collection Tubes	2 X 125 Nos.

Materials Required But Not Provided

- Water bath or Heat block
- Micropipettes
- Disposable barrier (Filter) pipette tips
- 1.5 ml micro-centrifuge tubes
- Table top Micro-centrifuge
- Ethanol (96-100%)
- Personal protection equipment (Aprons, gloves, goggles etc).
- Disposable gloves
- Mortar and Pestle

Instructions Before Use

Preheat a water bath or heating block to 56°C.

Wash BW1 and Wash BW2 are supplied as concentrates. Before using for the first time, add the appropriate volume of molecular biology grade ethanol (96-100%) as indicated on the bottle and shake thoroughly. Wash BW1 and Wash BW2 are stable for at least 18 months after the addition of ethanol when stored closed at room temperature (15-25°C).

Add protease dissolve buffer into absolute amount of Proteinase K as mentioned on the label and store it at -20°C.

Procedure

Isolation of Genomic DNA from Gram positive and Gram negative bacteria.

Protocol

A. DNA Purification from Bacteria














1) Collect 1.2 ml of an overnight bacterial broth culture in a 1.5 ml micro-centrifuge tube and centrifuge it for 1 min at 13,000xg at room temperature (15-25°C). Discard the supernatant.

2) Repeat the step 1 (If the volume of the pellet from the previous step is low).

3) Break the pellet by gentle finger tap.

4) Add 400 µl Buffer DLB to the pellet, 20 µl lysozyme solution and 5 µl RNaseA followed by incubation at room temperature (15-25°C) for 20 minutes.

- 5) Add 20 µl Proteinase K and 20 µl DLB-20 followed by incubation at 65°C for 30 minutes (for efficient lyses water bath is preferred).
- 6) Further add 0.5 volume of (96-100%) absolute ethanol into the solution and gently mix it by inverting the tube (A white precipitate may form on the addition of ethanol. It is essential to transfer all of the precipitate to the column).
- 7) Transfer the whole mixture on the gDNA Mini Column, centrifuge at 10,000xg for 1 min. Discard collection tube with flow-through.
- 8) Place the gDNA Mini Column into a fresh Collection Tube (2 ml) and add 500 µl Buffer W1. Centrifuge 1 min at 10,000xg. Discard flow-through.
- 9) Place the gDNA Mini Column back into the collection Tube (2ml) and add 500 µl Buffer W2. Centrifuge for 1 min at 10,000xg. Discard the flow-through.
- 10) Repeat step 9.
- 11) Dry spin: Centrifuge the Mini Column one more time at full speed or 20,000xg for 2 min.
- 12) Place the column into a fresh 1.5 ml micro-centrifuge tube and apply 50°C warmed 30-50 µl Buffer EB directly to the center of the silica membrane. Incubate at room temperature (15-25°C) for 3-5 min. Centrifuge at 10,000xg for 1 min.
- 13) Store the purified DNA at -20°C for long-term storage.

Symbols for Use in the Labeling	
Symbols	Definition
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED



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