RUO

REF	G2MBR4-0644	50 Tests
	G2MBR4-0645	250 Tests

SPINXT Bacterial RNA Extraction Kit

Intended Use

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

Summary

SpiNXT Bacterial RNA Extraction kit is designed for the rapid preparation of genomic RNA from 2×10^9 viable bacterial cells. Purification is based on silica membrane based spin columns as the separation matrix. It allow isolation of gRNA 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 RNA 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 manufac turing.
- 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.

Reagents Provided

Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0644
Buffer RLB	G2MBR3-1984-1	1 X 12 ml
Buffer RLB-1	G2MBR3-1985-1	1 X 25 ml
Buffer RLB-5	G2MBR3-1986-1	1 X 8 ml
Buffer W1	G2MBR3-1987-1	1 X 20 ml
Buffer W2	G2MBR3-1988-1	1 X 20 ml
Proteinase K	G2MBR3-1989-1	1 X 20 mg
Protease Dissolve Buffer	G2MBR3-1990-1	1 X 3 ml
Lysozyme	G2MBR3-1991-1	1 X 1 ml
Buffer EB	G2MBR3-1992-1	1 X 10 ml

Consumables Provided

Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0644
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 G2MBR4-0645
Buffer RLB	G2MBR3-1984-2	1 X 60 ml
Buffer RLB-1	G2MBR3-1985-2	1 X 110 ml
Buffer RLB-5	G2MBR3-1986-2	1 X 35 ml
Buffer W1	G2MBR3-1987-2	1 X 90 ml
Buffer W2	G2MBR3-1988-2	2 X 45 ml
Proteinase K	G2MBR3-1989-2	1 X 120 mg
Protease Dissolve Buffer	G2MBR3-1990-2	1 X 7 ml
Lysozyme	G2MBR3-1991-2	1 X 5 ml
Buffer EB	G2MBR3-1992-2	1 X 30 ml

Consumables Provided

Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0645
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.

Protocol

A. RNA Purification from Bacteria

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

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

3). Break the pellet by gentle finger tap.

4). Add 200 µl Buffer RLB to the pellet followed by 400µl Buffer RLB-1and 120 µl RLB-5. Ensure even mixing of all the buffers by gently tapping to the tube for 5-10 sec after addition of each buffer. (Avoid pooling of all the three buffers as it may result giving low yields.)

5). Add 20µl proteinase K and 20 µl lysozyme solution followed by incubation at 65°C for 30 min (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 for 1 minute (A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the column).

7). Pipette the mixture on the Mini Column and centrifuge it at 10,000 x*g* for 1 min. Discard collec-tion tube with flow-through.

8). Transfer the remaining lysate on to the column and again centrifuge it at 10,000 xg for 1 min. Discard collection tube with flow-through.

9). Place the Mini Column into a fresh Collection Tube (2ml) and add 500 µl Buffer W1. Centrifuge 1 min at 10,000 x*g*. Discard flow-through.

10). Place the Mini Column back into the collec-tion Tube (2ml) and add 500 µl Buffer W2. Centri-fuge1 min at 10,000 x*g*. Discard flow-through.

11). Repeat step 10.

12). Dry spin: Centrifuge the tube one more time at full speed or 20,000 x*g* for 2 min.
13). Place the column into a fresh 1.5 ml micro-centrifuge tube and apply 50°C warmed 30-50µl Buffer EB directly to the center of the silica membrane. Incubate at room temperature (15-25°C) for 3-5min. Centrifuge at 10,000 x*g* for 1min.

14). Store the purified RNA at -20°C for long-term storage.

Optional Steps

Add DNaseI after purification of high quality RNA, if needed to remove trace amounts of DNA to prevent contamination for further downstream processes (Usage as per the manufacturer's instructions).

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

Genes 2Me Private Limited Plot No - 33 Sector-5, IMT Manesar, Gurugram, Haryana - 122052 (India), Telephones No.: +91 18001 214030 / +91 88000 23600 / +91 8800821778, Email: contact@genes2me.com