

NUCQX® Magnetic Bacterial/Fungal DNA Kit

Cat. no. LM102103

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Product Information

Product name	NUCQX® Magnetic Bacterial / Fungal DNA Kit
Cat.No.	LM102103
Size	24T/48T
Storage and shipping	Part I: Proteinase K and RNase A, Store at 2 ~ 8°C. Transport at room temperature. Shelf life: 18 months. Part II: Store and transport at room temperature away from light.. Shelf life: 18 months.

2. Product Description

The NUCQX® Magnetic Bacterial/Fungal DNA Kit offers a simple and rapid solution for extracting DNA from Gram-negative bacteria, Gram-positive bacteria, and fungi. This method utilizes super paramagnetic bead purification technology and eliminates the need for toxic phenol-chloroform extraction. The procedure is straightforward, and the purified DNA can be directly used for restriction digestion, PCR, sequencing, and labeling.

3. Components

Comp.	Comp. No.	Comp. Name	LM102103 (24 T)	LM102103 (48 T)
Part I	LM102103-A	Proteinase K	0.6 mL/vial ×1	1.1 mL/vial ×1
	LM102103-B	RNase A	3 mg/vial ×1	6 mg/vial ×1
Part II	LM102103-C	RNase A Solvent	0.5 mL/vial ×1	1 mL/vial ×1
	LM102103-D	Lysis Buffer	13 mL/bottle ×1	25 mL/bottle ×1
	LM102103-E	Lysis/Binding Buffer	17 mL/bottle ×1	33 mL/bottle ×1
	LM102103-F	Magnetic Bead Suspension	0.6 mL/vial ×1	1.1 mL/vial ×1
	LM102103-G	Wash Buffer A	40 mL/bottle ×1	40 mL/bottle ×1
	LM102103-H	Wash Buffer B	20 mL/bottle ×1	40 mL/bottle ×1

LM102103-I	Wash Buffer C	20 mL/bottle ×1	40 mL/bottle ×1
LM102103-J	Elution Buffer	3 mL/bottle ×1	6 mL/bottle ×1
LM102103-K	Grinding Tubes	0.9 g × 24tubes	0.9 g × 48tubes

4. Safety and handling precautions

- Several buffers in this kit contain guanidine salts. Wear gloves and handle according to safety protocols. Avoid contact with skin, eyes, and mucosa. In case of contact, rinse thoroughly with water and seek medical attention. If any solution develops a precipitate, warm it in a 30°C water bath until the precipitate is completely dissolved before use.
- If precipitation occurs due to low temperature, incubate at 30°C to dissolve before use.
- If the lysis buffer precipitates, incubate at 60°C until fully dissolved.
- Do not use if the magnetic beads cannot be resuspended after vigorous shaking.
- Do not treat buffers containing guanidine salts with oxidizing disinfectants like sodium hypochlorite, as it may release toxic gases. Dispose of as medical waste.
- For research use only!

5. Instructions for Use

5.1. Reagent Preparation

- Before starting the experiment, check all solutions for precipitates and ensure that magnetic beads can be resuspended fully.
- Dissolving RNase A (10 mg/mL): Add 0.3 mL (24T)/0.6mL(48T) of RNase A Solvent to the RNase A vial to achieve a final concentration of 10 mg/mL. Invert or gently vortex until RNase A is fully dissolved. Store the dissolved RNase A at -20°C to 8°C.

5.2. Operating Procedure

Magnetic Separation Recommendation: After placing the centrifuge tube on the magnetic rack, gently rotate it side to side. Once the magnetic beads aggregate along the tube wall adjacent to the magnet, gently invert the magnetic rack several times to ensure that beads adhering to the tube cap also collect along the wall. Allow the tube to stand for several minutes until the solution becomes clear. The standing time may vary depending on the strength of the magnetic rack.

5.2.1. Sample Preparation

5.2.1.1. Transfer 0.5–1 mL of bacterial or fungal culture (approximately 10^6 – 10^9 cells) into a 1.5 mL microcentrifuge tube. Centrifuge at 10,000×g for 1 minute to pellet the cells and remove the supernatant as thoroughly as possible.

5.2.1.2. Choose the corresponding procedure based on the sample type:

A. DNA Extraction from Non-fungal and Easily Lysed Bacteria: Add 200–300 µL of lysis buffer and 20 µL

of Proteinase K, then vortex vigorously for 1 minute to mix thoroughly.

Easily lysed bacteria: Gram-negative bacteria, such as *Escherichia coli*, *Shigella*, *Salmonella*, *Proteus*, *Haemophilus influenzae*, *Legionella pneumophila*, *Bordetella pertussis*, *Vibrio cholerae*, etc.

B. DNA Extraction from Fungi and Hard-to-lyse Bacteria: Add 350-450 µL of lysis buffer and 20 µL of

Proteinase K, vortex briefly to mix, then centrifuge briefly to collect contents. Transfer the entire mixture to a grinding tube and vortex at maximum speed or transfer to a tissue homogenizer for high-speed disruption for 10-15 minutes (follow the manufacturer's recommended program for different homogenizer brands).

Fungi: Yeasts, *Candida*, *Aspergillus flavus*, *Geotrichum candidum*, *Actinomyces*, etc.

Hard-to-lyse bacteria: Gram-positive bacteria, such as *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Streptococcus pneumoniae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Clostridium tetani*, etc.

5.2.1.3. Incubate the samples in a shaking water bath at 65 °C for 15-30 minutes. If the constant-temperature device lacks a shaking function, remove the tubes every 5 minutes during incubation and vortex for 10-20 seconds each time.

5.2.1.4. Add 10µL of RNase A to the digestion mixture, vortex to mix thoroughly, and incubate at room temperature for 10-15 min. (If excessive sample volume leads to residual RNA, it is recommended to reduce the sample amount.)

5.2.1.5. If the lysate appears turbid after lysis, vortex to mix and centrifuge at 10,000 × g for 1 min. If the solution is clear and transparent, simply vortex to mix and perform a brief centrifugation.

Note: For fungi such as *Trichoderma* species, the supernatant may appear colored after centrifugation. In such cases, consult our technical support for a precipitation reagent and proceed as follows: transfer 300 µL of the supernatant to a new centrifuge tube, add 100 µL of the precipitation reagent, mix thoroughly, incubate on ice at 4 °C for 10 minutes, and centrifuge at 10,000 × g for 3 minutes.

5.2.2. Single-Tube Procedure

5.2.2.1. Transfer the supernatant from Step 1.5 into a new 1.5 mL microcentrifuge tube. Carefully aspirate to avoid disturbing the precipitate (which may result in RNA contamination).

5.2.2.2. Add 600 µL of Lysis/Binding Buffer. Vortex vigorously for 10 seconds to mix thoroughly.

5.2.2.3. Add 20µL of Magnetic Bead Suspension. Shake/vortex for 5-7 minutes to allow the magnetic beads to bind DNA.

5.2.2.4. Place the tube on the magnetic rack and allow magnetic separation until the solution becomes clear. Discard the supernatant.

5.2.2.5. Add 700 µL of Wash Buffer A, incubate for 30 seconds, then vortex for 10 seconds to resuspend the beads. Transfer to the magnetic rack and separate until the solution becomes clear. Discard the supernatant.

5.2.2.6. Add 700 µL of Wash Buffer B, vortex for 10 seconds to resuspend the beads, then place on the magnetic rack until the solution becomes clear. Discard the supernatant.

5.2.2.7. Add 700 µL of Wash Buffer C, vortex for 10 seconds to resuspend the beads, then place on the magnetic rack until the solution becomes clear. Discard the supernatant.

5.2.2.8. Briefly centrifuge the tube using a palm centrifuge, then return it to the magnetic rack until the solution becomes clear. Thoroughly remove any residual liquid. Open the tube cap and air-dry for 3–5 minutes.

Note: Residual ethanol may inhibit subsequent enzymatic reactions. Ensure complete ethanol evaporation during drying. Do not over-dry, as this may affect the elution efficiency.

5.2.2.9. Add 40–100 µL of Elution Buffer and vortex vigorously for 2–3 minutes to fully resuspend the magnetic beads.

5.2.2.10. Incubate at 60°C for 5 min, then vortex at high speed for 30 s. Repeat the elution once to increase yield if desired.

5.2.2.11. Briefly centrifuge to collect any droplets from the tube cap into the bottom of the tube. Place the tube onto the magnetic rack and separate until the solution becomes clear.

5.2.2.12. Transfer the genomic DNA solution to a new microcentrifuge tube for immediate use. If not used promptly, store at -15 ~ -25°C; for long-term storage, keep at -70°C or below.

5.2.3. Operation with the Medino® AP-16S and AP-48 Nucleic Acid Extraction Instruments

5.2.3.1. Dispense the reagents from the provided bottles into the corresponding wells of a 96-well plate according to the table below: Insert the DNA Binding Column T1 into a 2 mL collection tube and set aside.

Column	Steps	Reagent Name	Volume
1/7	Binding	Lysis/Binding Buffer	600 µL
2/8	-	-	-
3/9	Wash 1	Wash Buffer A	800 µL
4/10	Wash 2	Wash Buffer B	800 µL
5/11	Elution	Elution Buffer	100 µL
6/12	Wash 3	Magnetic Bead Suspension	20 µL (mix well before addition)
		Wash Buffer C	800 µL

5.2.3.2. Add the supernatant in Step 1.5 into the sample processing wells (columns 1 and 7) and mix by pipetting up and down 3–5 times.

5.2.3.3. Place the pre-loaded 96-well plate correctly into the extraction instrument, and insert the 8-rod magnetic bar sleeve (Cat#LM183663). Depending on the instrument model (AP-16S or AP-48), start the corresponding extraction program.

5.2.3.4. Run the program. After the program finishes, the solution in the elution wells (columns 5 and 11) is the nucleic acid eluate. The eluate may be stored at -20°C for short-term preservation and at -80°C for long-term storage.

AP-16S Nucleic Acid Extraction Instrument Program

Steps	1	2	3	4	5	6	7
Position	6	1	3	4	6	5	6
Waiting Time	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:01:00	0:00:00

Mixing Mode	M3	M3	M3	M3	M3	M4	M3
Mixing Time	0:00:30	0:10:00	0:01:00	0:01:00	0:01:00	0:05:00	0:00:30
Pause	No	No	No	No	No	No	No
Magnetic Separation Time	0:00:30	0:03:00	0:01:00	0:01:00	0:01:00	0:03:00	0:00:00
Volume (µL)	400	900	800	800	800	100*	800
Temperature	--	0°C	--	--	--	70°C	--
Mixing Mode	M3	Mixing time 10s, mixing speed 18,000 (rpm or instrument-specific units).					
Mixing Mode	M4	Mixing time 10s, mixing speed 23,000 (rpm or instrument-specific units).					

*Note: The elution volume (Step 6) can be adjusted according to the actual volume of Elution Buffer added.

AP-48 Nucleic Acid Extraction Instrument Program

Steps	1	2	3	4	5	6	7
Position	6	1	3	4	6	5	6
Waiting Time	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:01:00	0:00:00
Mixing Mode	M3	M3	M3	M3	M3	M4	M3
Mixing Time	0:00:30	0:10:00	0:01:00	0:01:00	0:01:00	0:05:00	0:00:30
Pause	No	No	No	No	No	No	No
Magnetic Separation Time	0:00:30	0:03:00	0:01:00	0:01:00	0:01:00	0:03:00	0:00:00
Volume (µL)	400	900	800	800	800	100*	800
Temperature	--	0°C	--	--	--	70°C	--
Mixing Mode	M3	Mixing time 10 s, mixing speed 300,000 (instrument-defined units)					
Mixing Mode	M4	Mixing time 10 s, mixing speed 400,000 (instrument-defined units)					

*Note: The elution volume (Step 6) can be adjusted according to the actual volume of Elution Buffer added.

5.2.4. Operation with the Medino® AP-96N Nucleic Acid Extraction Instruments

5.2.4.1. Dispense the Reagents into the Corresponding Wells of a 96-Well Plate (Cat#LM183671)

Plate Position	Step	Reagent Name	Volume
Plate 1	Binding	Lysis/Binding Buffer	600 µL
Plate 2	—	—	—
Plate 3	Wash 1	Wash Buffer A	800 µL
Plate 4	Wash 2	Wash Buffer B	800 µL
Plate 4Plate 5	Wash 3	Wash Buffer C	800 µL
		Magnetic Bead Suspension	20 µL (mix before addition)

Plate 6	Elution	Elution Buffer	100 µL
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5.2.4.2. Transfer all of the prepared lysate from Step 1.1 into the Plate 1 well (Lysis/Binding Plate) and pipette up and down 3–5 times, taking care to avoid undigested tissue fragments.

5.2.4.3. Place the loaded 96-well plates into the nucleic acid extraction instrument in order, and insert the 96-deep-well magnetic rod sleeve (Cat#LM183661).

5.2.4.4. Run the program. After program completion, transfer the eluate from the Elution Plate (Plate 6) into a new microcentrifuge tube. The eluate can be stored at -20°C for short-term preservation or at -80°C for long-term storage.

AP-96N Nucleic Acid Extraction Instrument Program

Steps	1	2	3	4	5	6	7
Position	5	1	3	4	5	6	5
Waiting Time	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:01:00	0:00:00
Mixing Mode	M3	M3	M3	M3	M3	M4	M3
Mixing Time	0:00:30	0:10:00	0:01:00	0:01:00	0:01:00	0:05:00	0:00:30
Pause	No	No	No	No	No	No	No
Magnetic Separation Time	0:00:30	0:03:00	0:01:00	0:01:00	0:01:00	0:03:00	0:00:00
Volume (µL)	400	900	800	800	800	100*	800
Temperature	--	0°C	--	--	--	70°C	--
Mixing Mode	M3	Mixing time 10 s, mixing speed 300,000 (instrument-defined units)					
Mixing Mode	M4	Mixing time 10 s, mixing speed 400,000 (instrument-defined units)					

*Note: The elution volume can be adjusted according to the actual volume of Elution Buffer added.