

Magnetic Plasmid Mini Kit

Cat. No.: M1521621 | Pack size: 48T | Storage: 2-8°C; room temperature; -20°C for designated component(s)

Overview

This kit is designed for the extraction of up to 20 µg of plasmid DNA from 1–5 mL of bacterial culture. The method employs superparamagnetic particle purification technology. The extraction process eliminates the need for toxic phenol-chloroform extraction. The procedure is simple and convenient, and the purified plasmid DNA can be used directly in downstream applications such as transformation, restriction enzyme digestion, PCR, first-generation sequencing, and library screening. The processed products are intended for scientific research and testing purposes only.

Kit Contents & Storage

Cat. No.	Component	48T	Storage
M1521621A	RNase A	2.5 mg	-20°C
M1521621B	Magnetic Beads MXVB	1.1 mL	2-8°C
M1521621C	Binding Buffer Bind	22 mL	RT.
M1521621D	Resuspension Buffer S1	14 mL	RT.
M1521621E	Lysis Buffer S2	14 mL	RT.
M1521621F	Precipitation Buffer N3	14 mL	RT.
M1521621G	Wash Buffer BW1	40 mL	RT.
M1521621H	Wash Buffer DW2	75 mL	RT.
M1521621I	Elution Buffer DE	6 mL	RT.

Each component has a shelf life of 12 months under corresponding storage conditions.

Storage Conditions

M1521621A: Store at -20°C, stable for 12 months.

M1521621B: Store at 2–8°C, stable for 12 months.

M1521621C, M1521621D, M1521621E, M1521621F, M1521621G, M1521621H, M1521621I: Store at room temperature, stable for 12 months.

Compatible Instruments

Manual extraction.

Precautions

Read this manual carefully before use.

Several buffers in this kit contain irritating guanidinium salts. Always wear gloves and handle according to standard safety precautions. Avoid contact of buffers with skin, eyes, and mucous membranes. In case of contact, flush immediately with plenty of water and seek medical attention.

If precipitation occurs in the solution due to low ambient temperatures, incubate at 30°C in a water bath until the precipitate is completely dissolved before use.

If precipitation occurs in Lysis Buffer S2 due to low ambient temperatures, incubate at 37°C in a water bath until the precipitate is completely dissolved before use.

If the magnetic beads cannot be resuspended after vigorous shaking due to low ambient temperatures, do not use the beads.

Several buffers in this kit contain guanidinium salts. Do not treat with oxidizing disinfectants such as sodium hypochlorite, as this may release toxic gases. Dispose of waste according to medical waste regulations.

This kit components are designed to be used together as a system. Components from different lot numbers should not be interchanged or mixed.

Instructions for Use

1. Sample Requirements

Applicable sample type: 1–5 mL bacterial culture.

Sample storage and transport: Samples may be processed immediately or stored at -70°C or lower for up to 6 months. Avoid repeated freeze-thaw cycles. Transport samples using cold chain logistics.

Freeze-thaw requirements: Rapid freezing and rapid thawing are recommended; avoid repeated freeze-thaw cycles.

2. Equipment and Materials to be Supplied by User

Constant temperature water bath (for use when precipitation occurs in solutions);

Dry block heater;

Magnetic separation rack (for 1.5/2.0 mL tubes);

1.5/2.0 mL sterile, nuclease-free centrifuge tubes;

Pipettes and tips of various volumes (20, 200, 1000 μ L).

3. Reagent Preparation

Check solutions for any precipitation and confirm that the magnetic beads can be resuspended.

Add 0.5–1 mL of Resuspension Buffer S1 to the RNase A dry powder vial. Mix by inversion or gentle vortexing to fully dissolve the RNase A. Transfer the entire RNase A solution back into the Resuspension Buffer S1 bottle and mix well. Store at 2–8°C; stable for 6 months after reconstitution.

4. Operating Procedure

Recommendation for magnetic separation (starting from step 4.9): After placing the centrifuge tube on the magnetic rack, gently rotate it left and right. Once the magnetic beads have aggregated against the tube wall closest to the magnet, gently invert the entire rack several times to collect any beads from the cap onto the tube wall. Allow to stand for several minutes until the solution becomes clear (standing time depends on the strength of the magnetic rack).

4.1 Add 1–5 mL of LB medium containing the appropriate antibiotic to a 10–20 mL culture tube. Inoculate the medium with the bacterial strain harboring the target vector and incubate at 37°C with shaking for 12–16 hours.

Note: The use of rich media such as TB or YT for bacterial culture is not recommended. Using rich media may result in incomplete removal of RNA.

4.2 Centrifuge at 10,000 $\times$ g for 1 minute to pellet 1–5 mL of bacterial cells.

4.3 Discard the supernatant completely. Gently tap the inverted tube on absorbent paper to remove residual liquid. Add 250 μ L of the prepared S1/RNase A mixture and resuspend the bacterial pellet thoroughly by high-speed vortexing.

Note: It is essential to completely resuspend the bacterial pellet. Incompletely dispersed clumps will affect lysis efficiency, leading to reduced yield and purity.

4.4 Add 250 μ L of Lysis Buffer S2 to the resuspended cells. Mix by gently inverting the tube 8–10 times. Incubate at room temperature for 1–2 minutes.

Note: Mix gently by inversion. Vortexing will cause genomic DNA contamination. The solution should become viscous and translucent, indicating complete bacterial lysis. If lysis with S2 is incomplete, it is recommended to reduce the initial amount of bacterial culture used.

4.5 Immediately add 250 μ L of Precipitation Buffer N3. Mix by gently inverting the tube 8–10 times to thoroughly neutralize the solution.

Note: Mix immediately upon adding N3 to prevent localized precipitation that may hinder neutralization. When larger volumes of bacterial culture are used, neutralization may be more difficult; invert with slightly more force several times until the solution is completely neutralized. The precipitate after neutralization should appear white. If neutralization is incomplete, increase the number of inversion cycles until fully neutralized.

4.6 Centrifuge at $10,000 \times g$ for 5 minutes.

4.7 Transfer 600 μL of the supernatant to a new 1.5 mL centrifuge tube.

4.8 Add 400 μL of Binding Buffer Bind and 20 μL of Magnetic Beads MXVB. Shake or vortex for 5–7 minutes to allow DNA binding to the beads.

4.9 Place the tube on the magnetic rack for magnetic separation until the solution is clear, then aspirate and discard the supernatant.

4.10 Add 700 μL of Wash Buffer BW1. Allow to soak for 30 seconds, then vortex to disperse the beads. Place on the magnetic rack for separation until the solution is clear. Aspirate and discard the supernatant.

4.11 Add 700 μL of Wash Buffer DW2. Vortex for 30 seconds to disperse the beads, then place on the magnetic rack for separation until the solution is clear. Aspirate and discard the supernatant.

4.12 Add another 700 μL of Wash Buffer DW2. Vortex for 30 seconds to disperse the beads, then place on the magnetic rack for separation until the solution is clear. Aspirate and discard the supernatant.

4.13 Perform a quick spin using a mini centrifuge, then place the tube back on the magnetic rack and allow beads to collect until the solution is clear. Carefully aspirate and discard all residual liquid. Open the tube cap and air-dry the beads for 3–5 minutes.

Note: Residual ethanol will inhibit downstream enzymatic reactions. Ensure ethanol has completely evaporated during drying. Do not over-dry the beads, as this may affect subsequent elution efficiency.

4.14 Add 50–80 μL of Elution Buffer DE and vortex at high speed for 2–3 minutes to fully disperse the beads.

4.15 Incubate at 60°C for 3–5 minutes, then vortex at high speed for 30 seconds.

4.16 Briefly centrifuge to collect any droplets from the cap into the tube, then place on the magnetic rack and allow to stand for 1–3 minutes.

4.17 Transfer the plasmid DNA solution to a new centrifuge tube for subsequent use. If not used immediately, store at -15 to -25°C . For long-term storage, place at -70°C or lower.

Limitations of the Method

For certain poorly collected samples, trace impurities in the extracted product may inhibit PCR amplification.

The efficiency of nucleic acid extraction is closely related to the quality of sample collection, handling, transport, and storage. Any error in these processes may lead to inaccurate results.

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