MagBeads Plasmid Maxi Preparation Kit

Introduction

Alkaline lysis method separates chromosomal DNA from plasmid DNA based on differences in DNA denaturation and renaturation, making it a widely used method for plasmid DNA preparation. The magnetic bead-based plasmid purification kit uses an improved SDS alkaline lysis method combined with a dual magnetic bead-based plasmid DNA extraction technique. This approach enables stable, efficient, and convenient plasmid DNA extraction. The kit avoids the need for high-speed centrifugation or vacuum filtration, allowing for rapid removal of impurities and specific binding of plasmid DNA.

This kit is suitable for extracting plasmid DNA from 50-200 mL overnight cultures of *E. coli* and can be used for downstream molecular biology experiments such as DNA sequencing, PCR, cell transfection, in vitro transcription, and restriction enzyme digestion. It supports both manual operation and automated extraction using nucleic acid extraction instruments for high-throughput automation.

Components and Storage

Components \ Size	K1627-10T	Storage
Buffer 1	50 mL	Room Temperature
Buffer 2	50 mL	Room Temperature
Buffer 3	70 mL	Room Temperature
Buffer B1	35 mL	Room Temperature
MagBeads	30 mL	4℃
Buffer W1	60 mL	Room Temperature
Buffer EB	20 mL	Room Temperature
RNase A	1 mL	-20℃
Shipping: Blue Ice	**Shelf life:** 12 months	

Material preparation

--Isopropanol; 80% ethanol; Vortex mixer; Magnetic rack; Pipette; Centrifuge; Vertical mixer; Water bath.

Preparation before first use

1. Add all the RNase A provided in the kit to Buffer 1, mix thoroughly, and store at 4℃.

2. Slowly add an equal volume of isopropanol to Buffer W1, mix thoroughly, and store at room temperature.

3. Before use, check Buffer 2 and Buffer 3 for any crystals or precipitates. If present, heat in a 37°C water bath for a few minutes to clarify.

4. Pre-warm the elution buffer in a 50°C water bath to improve plasmid DNA elution efficiency.

Reagent dosage

Components	Sample volume	
	50-100 mL	100-200 mL
Buffer 1	2 mL	4 mL
Buffer 2	2 mL	4 mL
Buffer 3	2.8 mL	5.6 mL
Buffer B1	1.4 mL	2.8 mL
Isopropanol	1.2 mL	2.4 mL
MagBeads	1.2 mL	2.4 mL
Buffer W1	3 mL	6 mL
80% ethanol	3 mL	6 mL
Buffer EB	500-800 µL	800 µL-1 mL

Protocol

(Example for extracting from 100 mL of bacterial culture)

1. Harvest Bacterial Cells

Collect 100 mL of overnight bacterial culture into a centrifuge tube, centrifuge at 12,000 rpm for 1 min, and discard the supernatant. Add 4 mL Buffer 1 (check if RNase A has been added), vortex until the bacterial pellet is completely resuspended.

2. Bacterial Cells Lysis

Add 4 mL Buffer 2 to the centrifuge tube, immediately gently invert the tube 8-10 times to mix, and let it sit at room temperature for 2 min to ensure complete lysis. The solution will become relatively viscous at this point.

***Note:** Mix gently to avoid vigorous shaking that could break the genomic DNA.

3. Remove gDNA and Proteins

3.1 Add 5.6 mL Buffer L3 to the centrifuge tube, gently invert 8-10 times to mix until a white, fluffy precipitate forms, then let it sit at room temperature for about 5 min.

3.2 Centrifuge at 10,000 rpm for 10 min to pellet the white precipitate.

3.3 Transfer the supernatant to a new centrifuge tube.

4. Plasmid Binding

4.1 To the new centrifuge tube, add 2.8 mL Buffer B1, 2.4 mL Isopropanol, and 2.4 mL MagBeads. Vortex for 10s, then place it on a vertical shaker for 5 min (or let it sit at room temperature, vortexing once every minute).

4.2 Place the centrifuge tube on magnetic rack for 2 min, then remove the supernatant with a pipette and remove the centrifuge tube.

5. Washing

5.1 Add 6 mL Buffer W1 (check if Isopropanol has been added), vortex for 1 min to resuspend the beads thoroughly. Place the centrifuge tube on the magnetic rack until the solution is clear, then remove the supernatant and remove the centrifuge tube.

5.2 Add 6 mL 80% ethanol, vortex for 1 min to resuspend the beads thoroughly, place the centrifuge tube on the magnetic rack until the solution is clear, then remove the supernatant and remove the centrifuge tube.

5.3 Repeat step **5.2** once.

***Note:** The final wash step should remove as much wash liquid as possible.

6. Drying

Keep the centrifuge tube on the magnetic rack and let it air-dry in a fume hood for 5 min.

***Note:** Ensure that the ethanol evaporates completely but avoid overdrying the beads to prevent cracking.

7. Elution

7.1 Add 1 mL elution buffer to the beads, vortex, or gently pipette up and down to resuspend the beads thoroughly.

***Note:** Before adding the elution buffer, it can be heated to 50°C in a water bath to improve plasmid DNA elution.

7.2 Place the centrifuge tube on the magnetic rack until the solution is clear, then transfer the supernatant to a new centrifuge tube and store at -20°C.

Note

1. If the bacterial culture volume is too large, it may cause the beads to clump, affecting washing efficiency and plasmid purity. If clumping occurs, try to disperse the beads during washing to improve extraction efficiency. Reducing the bacterial culture volume in future experiments is recommended.
2. Do not over-mix Buffer 2 to avoid excessive foaming. Close the bottle tightly after use to prevent CO_2 from the air from causing acidification.
3. The amount of extracted plasmid DNA is influenced by plasmid copy number and other factors. The OD value of the extracted plasmid DNA may vary slightly depending on the bacterial strain.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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