EcoSpin Plasmid Isolation Midi Kit

20 rxns

Cat No: MDP1050

Shipping : Ship at ambient temperature.
Storage : Store the Kit between 15°C and 25°C.
Store RNase A at -20°C

General Information

EcoSpin Plasmid Isolation Midi Kit is designed as a simple, convenient, and cost-effective purification of high-quality plasmid DNA from recombinant E. coli cultures. This kit utilizes chaotropic ions and silica-based membrane technology, eliminating the need for time-consuming alcohol precipitation method. The standard protocol lasts less than 60 minutes. The kit can be effectively used for purification of any size plasmids and cosmids. The relative plasmid yield and optimal culture size depend on the plasmid copy number and medium used for the bacterial culture.

Kit Contents

EcoSpin Resuspension Buffer	(60 ml)
EcoSpin Lysis Buffer	(60 ml)
EcoSpin Binding Buffer	(80 ml)
EcoSpin Equilibration Buffer	(25 ml)
EcoSpin Wash Buffer 1*	(48 ml)
EcoSpin Wash Buffer 2**	(25 ml concentrate)
EcoSpin Elution Buffer	(25 ml)
EcoSpin RNase A#	(lyophilized, 2 tubes)
EcoSpin Columns	(20)
EcoSpin Collection Tubes	(20)

*Add 32 ml absolute ethanol
**Add 100 ml absolute ethanol
Reconstitute each vial of RNase A in 1.1 ml Resuspension Buffer. RNase A solution is stable for 1 year when stored at 4ºC. For long-term storage (>1 year) store RNase A solution at -20ºC.

Protocol for Plasmid Isolation

Each isolation procedure is suitable for isolation of plasmid DNA from 20-50 ml of E. coli culture with an optical density of 1.5-5 at 600 nm. Bacterial culture should be inoculated using a single colony from a freshly streaked selective plate to an LB medium containing the appropriate selection antibiotic. The use of bacterial cultures grown for 12-16 hours at 37°C while shaking at 200-250 rpm are recommended.

1. Harvest the bacterial culture by centrifugation at 6000 rpm in a centrifuge for 5 minutes at room temperature. Decant or aspirate and discard the culture media.

2. Resuspend the bacterial pellet in 2.5 mL of the *EcoSpin* Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain. Add 100 µL of *EcoSpin* RNase A to the resuspended mixture. Transfer the cell suspension to a 15 mL or 50 mL centrifuge tubes capable of withstanding 12000 rpm.

3. Add 2.5 mL *EcoSpin* Lysis Buffer and mix gently by inverting the tube 6–7 times. Do not vortex to avoid shearing of genomic DNA. Incubate at room temperature for 3 minutes.

4. Add 3.5 mL *EcoSpin* Binding Buffer and mix thoroughly by inverting the tube 6–7 times. Do not vortex to avoid shearing of genomic DNA.

5. Centrifuge for 5 minutes at maximum speed at room temperature.

6. Add 1 mL *EcoSpin* Equilibration Buffer to the *EcoSpin* Midi Columns. Let sit at room temperature for 4 minutes. Centrifuge at 4000 rpm for 3 minutes. Discard the filtrate and reuse the collection tube.

7. Transfer 3.5 ml cleared supernatant from step 5 to the *EcoSpin* Midi Columns. Be careful not to disturb the pellet and that no cellular debris is transferred to the *EcoSpin* Midi Columns. Centrifuge at 4000 rpm for 3 minutes.

8. Discard the flowthrough and repeat Step 7 until all of the cleared supernatant has been transferred to the *EcoSpin* Midi Columns.

9. Discard the flowthrough and add 3.5 ml *EcoSpin* Wash Buffer 1 to the *EcoSpin* Midi Column. Centrifuge at 4000 rpm for 3 minutes at room temperature.

10. Discard the flowthrough and add 3.5 ml *EcoSpin* Wash Buffer 2 to the *EcoSpin* Midi Column. Centrifuge at 4000 rpm for 3 minutes at room temperature.

11. Discard the flowthrough and add 2 ml *EcoSpin* Wash Buffer 2 to the *EcoSpin* Midi Column. Centrifuge at 4000 rpm for 3 minutes at room temperature to completely remove any residual wash buffer.

12. Centrifuge the empty *EcoSpin* Midi Column at 4,000 rpm for 10 minutes to dry the column matrix.

13. Transfer the *EcoSpin* Midi Column to a clean 15 mL nuclease free centrifuge tube (not included). Add 0.3-1 mL of *EcoSpin* Elution Buffer to the center of the *EcoSpin* Midi Column membrane and incubate the column at room temperature for 3 minutes.

11. Centrifuge at 4000 rpm for 5 minutes at room temperature.

12. Discard the *EcoSpin* Midi Column and store the purified DNA at -20°C.

For further information;
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