EcoSpin Plasmid Isolation Kit

50 rxns

Cat No: EcoPI-50x

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 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 25 minutes and yields up to 20 µg of plasmid DNA. 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	(18 ml)
EcoSpin Lysis Buffer	(18 ml)
EcoSpin Binding Buffer	(22 ml)
EcoSpin Wash Buffer 1*	(13 ml)
EcoSpin Wash Buffer 2**	(8 ml concentrate)
EcoSpin Elution Buffer	(10 ml)
EcoSpin RNase A#	(lyophilized)
EcoSpin Columns	(50)
EcoSpin Collection Tubes	(50)

*Add 8.8 ml absolute ethanol
**Add 32 ml absolute ethanol
Reconstitute 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.

Additional Equipment & Reagents Required

96–100% ethanol
Tabletop microcentrifuge achieving >12,000 rpm
1.5 ml, sterile microcentrifuge tubes

Protocol for Plasmid Isolation

Each isolation procedure is suitable for isolation of plasmid DNA from 1-5 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 tabletop microcentrifuge for 2 minutes at room temperature. Discard the supernatant using a micropipette.

2. Resuspend the bacterial pellet in 250 µL of the *EcoSpin* Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain. Add 20 µL of *EcoSpin* RNase A to the resuspended mixture.

3. Add 250 µl *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 350 µL *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. Insert an *EcoSpin* Column into a Collection Tube and transfer the supernatant from step 5 to the *EcoSpin* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge for 30 seconds at room temperature.

7. Discard the flowthrough and add 400 µl *EcoSpin* Wash Buffer 1 to the *EcoSpin* Column. Centrifuge at maximum speed in a tabletop microcentrifuge for 30 seconds at room temperature.

8. Discard the flowthrough and add 500 µl *EcoSpin* Wash Buffer 2 to the *EcoSpin* Column. Centrifuge at maximum speed in a tabletop microcentrifuge for 30 seconds at room temperature.

9. Discard the flowthrough and add 200 µl *EcoSpin* Wash Buffer 2 *EcoSpin* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

10. Transfer the *EcoSpin* Column to a clean 1.5 mL microcentrifuge tube (not included). Add 30-50 µL of *EcoSpin* Elution Buffer to the center of the *EcoSpin* Column membrane and incubate the column at room temperature for 1 minute.

11. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

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

For further information;
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