

EcoSpin Plasmid Isolation MAXI Kit

Troubleshooting Guide

10 rxns

Cat No: MXP1050

Use this guide for common problems encountered during large-scale plasmid DNA purification from recombinant E. coli cultures. For best results, standardize culture conditions, fully resuspend the bacterial pellet, avoid vigorous mixing after lysis, clarify the lysate completely, equilibrate and load the Maxi Column as specified, use a swing bucket rotor for the column steps, prepare both wash buffers with ethanol, and complete the drying and elution steps.

Before You Start - Quick Checks

1. Use 50-200 mL recombinant E. coli culture with OD600 1.5-5; a 12-16 h culture at 37°C and 200-250 rpm is recommended.
2. Start from a single colony on a freshly streaked selective plate and use the appropriate selection antibiotic.
3. Add 36 mL absolute ethanol to EcoSpin Wash Buffer 1 and 160 mL absolute ethanol to EcoSpin Wash Buffer 2 before first use.
4. Reconstitute each RNase A vial in 1.1 mL Resuspension Buffer; add 300 µL RNase A after the bacterial pellet is completely resuspended.
5. Resuspend the pellet in 12 mL Resuspension Buffer until no clumps remain. After adding Lysis Buffer or Binding Buffer, mix only by gentle inversion; do not vortex.
6. Clarify for 10 min at maximum speed. For steps 6-12, use a swing bucket rotor as specified in the protocol.
7. Equilibrate the Maxi Column with 3 mL Equilibration Buffer for 4 min, centrifuge at 4000 rpm for 3 min, then load clarified lysate in repeated 14 mL portions.
8. Complete the 8 mL / 14 mL / 5 mL wash sequence, dry the empty column for 10 min at 4000 rpm, and elute with 1-3 mL Elution Buffer after a 3 min incubation.

Problem	Most likely cause	Recommended action
Low or no plasmid DNA yield	Culture volume or OD600 was outside the recommended range; the bacterial pellet was incompletely resuspended; clarification was incomplete; column equilibration or sample loading was not performed as specified; or not all cleared supernatant was loaded.	Use 50-200 mL E. coli culture with OD600 1.5-5. Resuspend the pellet completely in 12 mL Resuspension Buffer, follow the lysis/binding steps exactly, clarify for 10 min at maximum speed, equilibrate the Maxi Column with 3 mL Equilibration Buffer, and repeatedly load the cleared supernatant in 14 mL portions until all of it has passed through the column.
Variable plasmid yield between preparations	Culture volume, OD600, plasmid copy number, growth medium, growth time, shaking conditions, or column-loading completeness varied between preparations.	Standardize culture conditions: start from a single colony on a freshly streaked selective plate, use the appropriate selection antibiotic, grow 12-16 h at 37°C and 200-250 rpm, keep OD600 within 1.5-5, and process the complete clarified lysate consistently.
Bacterial pellet does not resuspend completely	The large bacterial pellet was compact or the 12 mL Resuspension Buffer was not mixed thoroughly through the pellet.	Resuspend the pellet completely in 12 mL EcoSpin Resuspension Buffer by vortexing or pipetting until no visible clumps remain. Add 300 µL RNase A only after a uniform suspension is obtained.
Genomic DNA contamination / viscous lysate	The sample was vortexed or mixed too aggressively after addition of Lysis Buffer or Binding Buffer, causing genomic DNA shearing and carryover.	After adding 12 mL Lysis Buffer, mix only by gently inverting 10-12 times. After adding 17 mL Binding Buffer, invert 6-7 times. Do not vortex during these two steps.
RNA contamination in plasmid preparation	RNase A was omitted, incorrectly reconstituted, or not added to the completely resuspended bacterial pellet.	Reconstitute each lyophilized RNase A vial in 1.1 mL Resuspension Buffer. Add 300 µL RNase A to the completely resuspended pellet before the lysis step.
Cleared lysate contains debris / column flow is poor	The 10 min clarification spin was shortened, the pellet was disturbed, or cellular debris was transferred to the Maxi Column.	Centrifuge for the full 10 min at maximum speed. Transfer only the cleared supernatant and do not disturb the pellet. The protocol specifically instructs that no cellular debris should be transferred to the Maxi Column.
Poor binding / DNA appears in flowthrough	Column equilibration was omitted or shortened, or the 3 mL Equilibration Buffer / 4 min incubation / 4000 rpm centrifugation conditions were not followed.	Add 3 mL EcoSpin Equilibration Buffer to the Maxi Column, let it sit for 4 min at room temperature, then centrifuge at 4000 rpm for 3 min and discard the filtrate before loading clarified lysate.
Low yield because not all lysate was processed	Only the first 14 mL portion of cleared supernatant was loaded and subsequent portions were not applied to the same column.	Load 14 mL cleared supernatant, centrifuge at 4000 rpm for 3 min, discard the flowthrough, and repeat until all cleared supernatant has been transferred to the Maxi Column.
Low yield or inconsistent flow during column steps	A rotor configuration other than the one specified for the Maxi column steps was used, or centrifugation conditions were inconsistent.	For steps 6-12, use a swing bucket rotor as stated in the protocol for maximum plasmid DNA yields and maintain the specified 4000 rpm centrifugation conditions.
Poor purity / downstream inhibition	Wash Buffer 1 or Wash Buffer 2 was not prepared with ethanol, or one of the specified wash steps was incomplete.	Before first use, add 36 mL absolute ethanol to Wash Buffer 1 and 160 mL absolute ethanol to Wash Buffer 2. Wash with 8 mL Wash Buffer 1, then 14 mL Wash Buffer 2, followed by 5 mL Wash Buffer 2 as specified.
Residual ethanol or wash buffer in eluate	The final 5 mL Wash Buffer 2 step or the additional 10 min empty-column drying spin was omitted or shortened.	After the 5 mL Wash Buffer 2 wash and 3 min centrifugation, centrifuge the empty Maxi Column at 4000 rpm for 10 min to dry the column matrix before elution.

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Problem	Most likely cause	Recommended action
Low plasmid concentration in eluate	A larger elution volume within the 1-3 mL range was used, or the buffer did not contact the center of the membrane.	For a more concentrated preparation, use the lower end of the 1-3 mL EcoSpin Elution Buffer range. Apply the buffer to the center of the membrane and incubate 3 min at room temperature before centrifugation.
No or very little eluate recovered	Elution Buffer did not fully contact the membrane, the 3 min incubation was skipped, or the final 4000 rpm centrifugation was incomplete.	Transfer the Maxi Column to a clean 50 mL nuclease-free tube, add 1-3 mL Elution Buffer to the membrane center, incubate for 3 min, then centrifuge at 4000 rpm for 5 min at room temperature.
Lower yield than expected from a low-copy plasmid	Plasmid copy number and culture medium can limit the amount of plasmid DNA available for purification.	Keep culture conditions within the specified 50-200 mL and OD600 1.5-5 ranges and use the recommended selective 12-16 h culture conditions. The datasheet notes that relative yield and optimal culture size depend on plasmid copy number and medium.
Poor reproducibility despite correct reagent volumes	Culture age, OD600, resuspension completeness, mixing intensity, clarification, rotor type, equilibration time, repeated loading, wash spins, drying, or elution conditions varied between runs.	Standardize the complete workflow from culture preparation through gentle lysis/binding, 10 min clarification, swing-bucket column processing, repeated 14 mL loading, specified washes, 10 min column drying, and 1-3 mL center-of-membrane elution.

Still having trouble? Confirm the culture volume and OD600, complete pellet resuspension, 300 µL RNase A addition, gentle inversion after Lysis and Binding Buffers, 10 min clarification, swing-bucket rotor use for the column steps, 3 mL Equilibration Buffer / 4 min equilibration, repeated 14 mL sample loading, Wash Buffer ethanol additions, the complete wash sequence, 10 min empty-column drying spin, and 1-3 mL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the plasmid/culture details, OD600, culture volume, centrifugation setup, reagent preparation, and observed yield or purity.