

EcoSpin Plasmid Isolation Mini Kit

Troubleshooting Guide

50 rxns

Cat No: EcoPI-50x

Use this guide for common problems encountered during plasmid DNA purification from recombinant E. coli cultures. For best results, standardize culture conditions, fully resuspend the bacterial pellet, avoid vigorous mixing after lysis, perform the column activation step exactly, prepare both wash buffers with ethanol, and follow the specified elution conditions.

Before You Start - Quick Checks

1. Use 1-5 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 8.8 mL absolute ethanol to EcoSpin Wash Buffer 1 and 32 mL absolute ethanol to EcoSpin Wash Buffer 2 before first use.
4. Reconstitute lyophilized RNase A in 1.1 mL Resuspension Buffer; add 20 µL RNase A after the bacterial pellet is completely resuspended.
5. Resuspend the pellet in 250 µL Resuspension Buffer until no clumps remain. After adding Lysis Buffer or Binding Buffer, mix only by gentle inversion; do not vortex.
6. After adding 350 µL Binding Buffer, centrifuge for 5 min at maximum speed and transfer only the clarified supernatant.
7. Activate each EcoSpin Column with 200 µL Activation Buffer for 4 min at room temperature, then centrifuge at 4000 rpm for 3 min before sample loading.
8. Elute with 30-50 µL EcoSpin Elution Buffer applied to the membrane center; incubate 1 min at room temperature before centrifugation.

Problem	Most likely cause	Recommended action
Low or no plasmid DNA yield	Culture density, plasmid copy number, or culture volume was outside the recommended range; the pellet was incompletely resuspended; lysis/Binding Buffer mixing was incomplete; or the activated column was not used as specified.	Use 1-5 mL E. coli culture with OD600 1.5-5 and a 12-16 h culture grown at 37°C with shaking at 200-250 rpm. Resuspend the pellet completely in 250 µL Resuspension Buffer, follow the lysis/binding steps exactly, activate the column with 200 µL Activation Buffer, and transfer the clarified supernatant to the activated column.
Variable plasmid yield between preparations	Culture volume, OD600, plasmid copy number, growth medium, or growth time varied between preparations.	Standardize culture conditions. Start from a single colony on a freshly streaked selective plate, use the appropriate selection antibiotic, grow for 12-16 h at 37°C and 200-250 rpm, and keep culture volume within the 1-5 mL range.
Bacterial pellet does not resuspend completely	Pellet was compact or Resuspension Buffer was not mixed thoroughly with the cells before lysis.	Resuspend the pellet in 250 µL EcoSpin Resuspension Buffer by vortexing or pipetting until no visible cell clumps remain. Add 20 µL RNase A only after a uniform suspension is obtained.
Genomic DNA contamination / high-molecular-weight DNA in eluate	The sample was vortexed or mixed too aggressively after addition of Lysis Buffer or Binding Buffer, causing genomic DNA shearing and carryover.	After adding 250 µL Lysis Buffer, mix gently by inverting the tube 6-7 times and do not vortex. Repeat gentle inversion after adding 350 µL Binding Buffer. Avoid harsh mixing during these steps.
RNA contamination in plasmid preparation	RNase A was omitted, not reconstituted correctly, or not added to the resuspended pellet.	Reconstitute lyophilized RNase A in 1.1 mL Resuspension Buffer as specified. Add 20 µL RNase A to the completely resuspended bacterial pellet before the lysis step.
Lysate remains cloudy or column clogs	The pellet was not fully resuspended before lysis, the sample was not sufficiently clarified, or particulate material was transferred after the 5 min maximum-speed centrifugation.	Ensure complete pellet resuspension before lysis. After Binding Buffer addition, centrifuge for the full 5 min at maximum speed and transfer only the clarified supernatant to the activated EcoSpin Column.
Poor binding / DNA appears in flowthrough	Column activation was omitted, Activation Buffer contact time was shortened, or the activation centrifugation condition was not followed.	Before loading the clarified lysate, add 200 µL EcoSpin Activation Buffer to the column, allow it to sit for 4 min at room temperature, then centrifuge at 4000 rpm for 3 min and discard the filtrate.
Column activation gives inconsistent results	Activation Buffer volume, 4 min incubation, or 4000 rpm / 3 min centrifugation was not kept consistent.	Use exactly 200 µL Activation Buffer per column, incubate 4 min at room temperature, centrifuge at 4000 rpm for 3 min, discard the filtrate, and then load the clarified supernatant.
Poor purity or downstream inhibition	Wash Buffer 1 or Wash Buffer 2 was not prepared with ethanol, washing was incomplete, or residual wash buffer remained on the membrane.	Before first use, add 8.8 mL absolute ethanol to Wash Buffer 1 and 32 mL absolute ethanol to Wash Buffer 2. Perform the 400 µL Wash Buffer 1 wash, 500 µL Wash Buffer 2 wash, and the final 200 µL Wash Buffer 2 / 2 min spin exactly as specified.
Residual ethanol / wash buffer in eluate	The final 200 µL Wash Buffer 2 step or the 2 min maximum-speed centrifugation was shortened or omitted.	After the 500 µL Wash Buffer 2 wash, add 200 µL Wash Buffer 2 and centrifuge the column for 2 min at maximum speed to remove residual wash buffer before elution.

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Problem	Most likely cause	Recommended action
Low plasmid concentration in eluate	The larger end of the 30-50 µL elution range was used, or the elution buffer was not applied to the center of the membrane.	For a more concentrated eluate, use 30 µL EcoSpin Elution Buffer. Apply it directly to the center of the membrane, incubate 1 min at room temperature, then centrifuge 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not fully contact the membrane, the 1 min incubation was skipped, or the final centrifugation was incomplete.	Transfer the column to a clean 1.5 mL tube, add 30-50 µL Elution Buffer to the membrane center, incubate for 1 min at room temperature, and centrifuge 30 sec at maximum speed.
Low yield from a low-copy plasmid	Plasmid copy number and culture conditions inherently limit the amount of plasmid DNA available for purification.	Keep the culture within the specified 1-5 mL and OD600 1.5-5 range and use the recommended 12-16 h selective culture conditions. Note that the datasheet states that relative yield depends on plasmid copy number and culture medium.
Poor reproducibility despite correct reagent volumes	Culture age, OD600, mixing intensity, clarification, activation time, centrifuge speed, or elution conditions varied between runs.	Standardize the entire workflow: culture conditions, complete resuspension, gentle inversion during lysis/binding, 5 min clarification, 4 min column activation, specified wash spins, and 30-50 µL center-of-membrane elution.

Still having trouble? Confirm the culture volume and OD600, complete pellet resuspension, 20 µL RNase A addition, gentle inversion after Lysis and Binding Buffers, 5 min clarification, 200 µL Activation Buffer / 4 min activation step, Wash Buffer ethanol additions, final 2 min wash spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the plasmid/culture details, OD600, culture volume, reagent preparation, centrifugation conditions, and observed yield or purity.