

# EcoSpin Plasmid Isolation Midi Kit

## Troubleshooting Guide

20 rxns

Cat No: MDP1050

Use this guide for common problems encountered during medium-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 Midi Column as specified, prepare both wash buffers with ethanol, and complete the drying and elution steps.

### Before You Start - Quick Checks

1. Use 20-50 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 32 mL absolute ethanol to EcoSpin Wash Buffer 1 and 100 mL absolute ethanol to EcoSpin Wash Buffer 2 before first use.
4. Reconstitute each RNase A vial in 1.1 mL Resuspension Buffer; add 100 µL RNase A after the bacterial pellet is completely resuspended.
5. Resuspend the pellet in 2.5 mL Resuspension Buffer until no clumps remain. After adding Lysis Buffer or Binding Buffer, mix only by gentle inversion; do not vortex.
6. Clarify the lysate for 5 min at maximum speed and transfer only the cleared supernatant without disturbing the pellet.
7. Equilibrate the Midi Column with 1 mL Equilibration Buffer for 4 min, centrifuge at 4000 rpm for 3 min, then load clarified lysate in repeated 3.5 mL portions.
8. Complete the 3.5 mL / 3.5 mL / 2 mL wash sequence, dry the empty column for 10 min at 4000 rpm, and elute with 0.3-1 mL Elution Buffer after a 3 min incubation.

| Problem                                                     | Most likely cause                                                                                                                                                                                                                                                | Recommended action                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Low or no plasmid DNA yield</b>                          | 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 processed. | Use 20-50 mL E. coli culture with OD600 1.5-5. Resuspend the pellet completely in 2.5 mL Resuspension Buffer, follow the lysis and binding steps exactly, clarify for 5 min at maximum speed, equilibrate the Midi Column with 1 mL Equilibration Buffer, and repeatedly load the cleared supernatant in 3.5 mL portions until all of it has passed through the column. |
| <b>Variable plasmid yield between preparations</b>          | Culture volume, OD600, plasmid copy number, growth medium, culture age, shaking conditions, or completeness of sample loading 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.                                                                                                      |
| <b>Bacterial pellet does not resuspend completely</b>       | The bacterial pellet remained compact or the 2.5 mL Resuspension Buffer was not mixed thoroughly through the pellet.                                                                                                                                             | Resuspend the pellet completely in 2.5 mL EcoSpin Resuspension Buffer by vortexing or pipetting until no visible clumps remain. Add 100 µL RNase A only after a uniform suspension is obtained.                                                                                                                                                                         |
| <b>Genomic DNA contamination / viscous lysate</b>           | The sample was vortexed or mixed too aggressively after addition of Lysis Buffer or Binding Buffer, causing genomic DNA shearing and carryover.                                                                                                                  | After adding 2.5 mL Lysis Buffer, mix only by gently inverting 6-7 times. After adding 3.5 mL Binding Buffer, again invert 6-7 times. Do not vortex during these steps.                                                                                                                                                                                                 |
| <b>RNA contamination in plasmid preparation</b>             | RNase A was omitted, incorrectly reconstituted, or not added after complete pellet resuspension.                                                                                                                                                                 | Reconstitute each lyophilized RNase A vial in 1.1 mL Resuspension Buffer. Add 100 µL RNase A to the completely resuspended bacterial pellet before the lysis step.                                                                                                                                                                                                      |
| <b>Cleared lysate contains debris / column flow is poor</b> | The 5 min clarification spin was shortened, the pellet was disturbed, or cellular debris was transferred to the Midi Column.                                                                                                                                     | Centrifuge for the full 5 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 Midi Column.                                                                                                                                              |
| <b>Poor binding / DNA appears in flowthrough</b>            | Column equilibration was omitted or shortened, or the 1 mL Equilibration Buffer / 4 min incubation / 4000 rpm centrifugation conditions were not followed.                                                                                                       | Add 1 mL EcoSpin Equilibration Buffer to the Midi 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.                                                                                                                                                                     |
| <b>Low yield because not all lysate was processed</b>       | Only the first 3.5 mL portion of cleared supernatant was loaded and subsequent portions were not applied to the same column.                                                                                                                                     | Load 3.5 mL cleared supernatant, centrifuge at 4000 rpm for 3 min, discard the flowthrough, and repeat until all cleared supernatant has been transferred to the Midi Column.                                                                                                                                                                                           |
| <b>Poor purity / downstream inhibition</b>                  | 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 32 mL absolute ethanol to Wash Buffer 1 and 100 mL absolute ethanol to Wash Buffer 2. Wash with 3.5 mL Wash Buffer 1, then 3.5 mL Wash Buffer 2, followed by 2 mL Wash Buffer 2 as specified.                                                                                                                                                     |
| <b>Residual ethanol or wash buffer in eluate</b>            | The final 2 mL Wash Buffer 2 step or the additional 10 min empty-column drying spin was omitted or shortened.                                                                                                                                                    | After the 2 mL Wash Buffer 2 wash and 3 min centrifugation, centrifuge the empty Midi Column at 4000 rpm for 10 min to dry the column matrix before elution.                                                                                                                                                                                                            |
| <b>Low plasmid concentration in eluate</b>                  | A larger elution volume within the 0.3-1 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 0.3-1 mL EcoSpin Elution Buffer range. Apply the buffer to the center of the membrane and incubate 3 min at room temperature before centrifugation.                                                                                                                                                       |

*Table continues on next page.*

## EcoSpin Plasmid Isolation Midi Kit Troubleshooting Guide (continued)

| Problem                                                     | Most likely cause                                                                                                                                                                           | Recommended action                                                                                                                                                                                                                                                        |
|-------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>No or very little eluate recovered</b>                   | Elution Buffer did not fully contact the membrane, the 3 min incubation was skipped, or the final 4000 rpm centrifugation was incomplete.                                                   | Transfer the Midi Column to a clean 15 mL nuclease-free tube, add 0.3-1 mL Elution Buffer to the membrane center, incubate for 3 min, then centrifuge at 4000 rpm for 5 min at room temperature.                                                                          |
| <b>Lower yield than expected from a low-copy plasmid</b>    | Plasmid copy number and culture medium can limit the amount of plasmid DNA available for purification.                                                                                      | Keep culture conditions within the specified 20-50 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.                     |
| <b>Poor reproducibility despite correct reagent volumes</b> | Culture age, OD600, resuspension completeness, mixing intensity, clarification, equilibration time, repeated loading, wash spins, column drying, or elution conditions varied between runs. | Standardize the complete workflow from culture preparation through complete resuspension, gentle lysis/binding, 5 min clarification, 1 mL column equilibration, repeated 3.5 mL loading, specified washes, 10 min column drying, and 0.3-1 mL center-of-membrane elution. |

Still having trouble? Confirm the culture volume and OD600, complete pellet resuspension, 100 µL RNase A addition, gentle inversion after Lysis and Binding Buffers, 5 min clarification, 1 mL Equilibration Buffer / 4 min equilibration, repeated 3.5 mL sample loading, Wash Buffer ethanol additions, the complete wash sequence, 10 min empty-column drying spin, and 0.3-1 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.