

EcoSpin PCR Purification Kit

Troubleshooting Guide

50 rxns

Cat No: EcoPP-50x

Use this guide for common problems encountered during PCR product cleanup. For best results, follow the specified PCR sample volume, Binding Buffer and isopropanol ratios, Wash Buffer preparation, centrifugation, dry-spin, and elution conditions in the product protocol.

Before You Start - Quick Checks

1. Add 32 mL absolute ethanol to the 8 mL EcoSpin Wash Buffer concentrate before first use.
2. Use 96-100% ethanol, 100% isopropanol, sterile 1.5 mL microcentrifuge tubes, and a tabletop microcentrifuge capable of >12,000 rpm.
3. The standard procedure is designed for 50 µL PCR product. Adjust samples below 50 µL to a total volume of 50 µL.
4. For PCR samples above 50 µL, increase Binding Buffer proportionally or divide the sample into 50 µL aliquots.
5. Add 250 µL EcoSpin Binding Buffer and 100 µL isopropanol to each 50 µL PCR product and mix well.
6. If primer-dimers are present, purification may be performed without isopropanol, but target DNA recovery may decrease.
7. Wash with 700 µL EcoSpin Wash Buffer, then centrifuge the empty column for an additional 1 min at maximum speed to remove residual wash buffer.
8. Elute with 30-50 µL EcoSpin Elution Buffer applied to the center of the membrane; incubate 1 min at room temperature before centrifugation.

Problem	Most likely cause	Recommended action
Low or no DNA recovery	PCR sample volume was not adjusted to 50 µL; Binding Buffer or isopropanol amount was incorrect; the complete sample was not transferred to the column; or centrifugation conditions differed from the protocol.	For each 50 µL PCR product, add 250 µL EcoSpin Binding Buffer and 100 µL isopropanol and mix well. For samples smaller than 50 µL, adjust the total volume to 50 µL. For samples larger than 50 µL, increase Binding Buffer proportionally or divide the sample into 50 µL aliquots.
Variable yield between PCR samples	Different PCR volumes were processed with the same reagent volumes, or sample loading, centrifugation, or elution conditions were not kept consistent.	Standardize each purification around a 50 µL PCR product. Scale Binding Buffer appropriately for larger samples, keep isopropanol addition consistent, and use the same wash, dry-spin, incubation, and final centrifugation conditions for all samples.
Primer-dimers remain in the purified DNA	The standard purification workflow with isopropanol was used even though the PCR mixture contained primer-dimers.	When primer-dimers are present, purification may be performed without isopropanol as stated in the protocol. Be aware that omitting isopropanol may reduce recovery of the target DNA fragment.
Target DNA yield decreases after primer-dimer removal	Isopropanol was intentionally omitted to reduce primer-dimer carryover.	This lower target-fragment yield is an expected trade-off described in the protocol. If maximum target recovery is the priority, use the standard workflow including 100 µL isopropanol per 50 µL PCR product.
Poor purity or downstream inhibition	Wash Buffer was not prepared with ethanol, the wash step was incomplete, or residual wash components remained on the membrane.	Before first use, add 32 mL absolute ethanol to the 8 mL EcoSpin Wash Buffer concentrate. Wash with 700 µL Wash Buffer, centrifuge for 30 sec at maximum speed, discard the flowthrough, and perform the additional 1 min empty-column spin.
Residual ethanol / wash buffer in the eluate	The additional empty-column centrifugation after washing was omitted, shortened, or not performed at maximum speed.	After the 700 µL wash, discard the flowthrough and centrifuge the empty EcoSpin Column for an additional 1 min at maximum speed before transferring it to a clean tube for elution.
Column flow is slow or sample remains on the membrane	A large sample was loaded without dividing it into aliquots, or centrifugation was below the protocol-specified maximum speed.	For PCR volumes larger than 50 µL, divide the sample into 50 µL aliquots or scale the Binding Buffer proportionally as instructed. Centrifuge for the specified time at maximum speed in a tabletop microcentrifuge.
Low DNA concentration in the eluate	Elution volume was at the high end of the specified range, Elution Buffer was not applied to the membrane center, or the 1 min incubation was omitted.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the membrane and incubate for 1 min at room temperature before the final 30 sec centrifugation. Use a consistent elution volume when comparing samples.
No or very little eluate recovered	The column was not transferred to a clean collection tube before elution, Elution Buffer did not contact the membrane adequately, or the final centrifugation was incomplete.	Transfer the column to a clean 1.5 mL microcentrifuge tube, add 30-50 µL Elution Buffer to the center of the membrane, incubate 1 min, and centrifuge for 30 sec at maximum speed.
Wash step gives inconsistent results	The 8 mL Wash Buffer concentrate was used without adding 32 mL absolute ethanol, or the 700 µL wash volume was not followed.	Prepare the Wash Buffer correctly before use by adding 32 mL absolute ethanol. Use 700 µL per column and centrifuge for 30 sec at maximum speed before the additional dry spin.

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Problem	Most likely cause	Recommended action
Poor recovery from PCR volumes below 50 µL	The protocol was started directly with a sample volume below 50 µL instead of adjusting the total volume to 50 µL.	Adjust each PCR sample below 50 µL to a total volume of 50 µL before adding 250 µL EcoSpin Binding Buffer and 100 µL isopropanol.
Poor recovery from PCR volumes above 50 µL	A PCR sample larger than 50 µL was processed using the standard 250 µL Binding Buffer volume without proportional scaling.	Increase the amount of Binding Buffer proportionally for samples above 50 µL, or divide the PCR product into 50 µL aliquots and process according to the standard protocol.
Unexpected contamination in downstream reactions	Residual salts, free nucleotides, Taq polymerase, or wash components were not completely removed because purification or wash steps were incomplete.	Follow the complete binding, 700 µL wash, and additional 1 min empty-column centrifugation sequence before elution. Use a clean 1.5 mL tube for the final elution.
Results remain inconsistent despite correct reagent volumes	Timing, centrifuge speed, elution placement, or incubation conditions varied between runs.	Repeat the workflow using maximum-speed centrifugation for the specified times, the full 1 min dry spin, 30-50 µL Elution Buffer applied to the membrane center, and a 1 min room-temperature incubation before the final spin.

Still having trouble? Confirm the 50 µL PCR starting volume or proportional reagent scaling, 250 µL Binding Buffer and 100 µL isopropanol per 50 µL PCR product, Wash Buffer ethanol addition, maximum-speed centrifugation, the additional 1 min empty-column spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the PCR volume, reagent volumes, centrifugation conditions, and observed DNA yield or downstream issue.