

EcoSpin PCR Purification Micro Kit

Troubleshooting Guide

50 rxns

Cat No: E2525

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

Before You Start - Quick Checks

1. Add 32 mL absolute ethanol to the 8 mL EcoSpin Micro 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 the Micro Binding Buffer proportionally or divide the sample into 50 µL aliquots.
5. Add 250 µL EcoSpin Micro 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 Micro Wash Buffer, then centrifuge the empty Micro Spin Column for an additional 1 min at maximum speed.
8. Elute with 10-20 µL EcoSpin Micro 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; Micro Binding Buffer or isopropanol amount was incorrect; the complete sample was not transferred to the Micro Spin Column; or the centrifugation step differed from the protocol.	For each 50 µL PCR product, add 250 µL EcoSpin Micro Binding Buffer and 100 µL isopropanol and mix well. For samples below 50 µL, adjust the total volume to 50 µL. For samples above 50 µL, increase Binding Buffer proportionally or divide the sample into 50 µL aliquots.
Variable yield between PCR samples	Different PCR sample volumes were processed with identical reagent volumes, or sample loading, centrifugation, dry-spin, and elution conditions were not kept consistent.	Standardize each purification around a 50 µL PCR product. Scale the Micro Binding Buffer for larger samples, keep isopropanol addition consistent, and use the same wash, dry-spin, 1 min elution incubation, and final centrifugation conditions for all samples.
Primer-dimers remain in the purified DNA	The standard purification workflow including isopropanol was used even though the PCR mixture contained primer-dimers.	If primer-dimers are present, purification may be performed without isopropanol as stated in the protocol. Note that omitting isopropanol may reduce the recovery of the target DNA fragment.
Target DNA recovery decreases when primer-dimer removal is prioritized	Isopropanol was intentionally omitted to reduce primer-dimer carryover.	This lower target-fragment recovery is the trade-off stated in the protocol. When target recovery is the priority, use the standard workflow with 100 µL isopropanol per 50 µL PCR product.
Poor purity or downstream inhibition	Micro Wash Buffer was used before ethanol was added, 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 Micro Wash Buffer concentrate. Wash with 700 µL, centrifuge 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 or shortened.	After the 700 µL wash, discard the flowthrough and centrifuge the empty EcoSpin Micro Spin 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 larger-than-standard PCR volume was loaded without scaling or aliquoting, or the centrifugation was not performed at maximum speed.	For PCR volumes above 50 µL, either scale the Micro Binding Buffer proportionally or divide the sample into 50 µL aliquots before loading. Perform the specified centrifugation steps at maximum speed.
Eluate concentration is lower than expected	The 20 µL end of the specified elution range was used when a smaller final volume was desired, or Micro Elution Buffer was not applied directly to the center of the membrane.	Use 10-20 µL EcoSpin Micro Elution Buffer according to the required final volume. Apply the buffer directly to the center of the membrane and incubate for 1 min at room temperature before the final 30 sec centrifugation.
No or very little eluate recovered	The Micro Spin Column was not transferred to a clean 1.5 mL tube before elution, the elution buffer did not fully contact the membrane, or the final centrifugation was incomplete.	Transfer the column to a clean 1.5 mL microcentrifuge tube, add 10-20 µL EcoSpin Micro Elution Buffer to the membrane center, incubate 1 min at room temperature, and centrifuge 30 sec at maximum speed.
Wash step gives inconsistent results	The 8 mL Micro Wash Buffer concentrate was used without adding 32 mL absolute ethanol, or the specified 700 µL wash volume was not followed.	Prepare the Micro Wash Buffer before use by adding 32 mL absolute ethanol. Use 700 µL per column and centrifuge 30 sec at maximum speed before the additional 1 min 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 PCR 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 Micro 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 Micro Binding Buffer volume without proportional scaling.	Increase the amount of Micro 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	Primer-dimers, free nucleotides, salts, Taq polymerase, or wash components were not sufficiently removed because the purification or wash sequence was incomplete.	Follow the complete binding, 700 µL wash, additional 1 min empty-column centrifugation, and center-of-membrane elution sequence. Use a clean 1.5 mL tube for the final eluate.
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, 10-20 µL Micro 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 Micro Binding Buffer and 100 µL isopropanol per 50 µL PCR product, Micro Wash Buffer ethanol addition, maximum-speed centrifugation, the additional 1 min empty-column spin, and 10-20 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the PCR volume, reagent volumes, centrifugation conditions, elution volume, and observed DNA yield or downstream issue.