

EcoSpin Gel Purification Kit

Troubleshooting Guide

50 rxns

Cat No: EcoGP-50x

Use this guide for common problems encountered during recovery of DNA from agarose gels. For best results, follow the specified gel-mass-to-buffer ratios, complete agarose dissolution, isopropanol addition, wash and dry-spin steps, 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. Run the PCR product on a 0.8-2% agarose gel in 1x TAE or 1x TBE until the desired DNA band is clearly separated from adjacent fragments.
4. Excise the desired band using an ethanol-cleaned scalpel or razor blade and determine the gel mass accurately by re-weighing the tube.
5. Add 300 µL EcoSpin Binding Buffer per 100 mg agarose. For larger gel slices, increase the Binding Buffer proportionally.
6. Dissolve the gel for 10 min at 55°C, vortexing briefly every 2-3 min. After complete dissolution, add 150 µL isopropanol per 100 mg gel.
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 membrane center; incubate 1 min at room temperature before centrifugation.

Problem	Most likely cause	Recommended action
Low or no DNA recovery from the gel slice	Gel mass was not matched to Binding Buffer and isopropanol volumes; agarose was not completely dissolved; or sample transfer to the column was incomplete.	Weigh the excised gel slice accurately. For each 100 mg agarose, add 300 µL EcoSpin Binding Buffer, dissolve completely at 55°C for 10 min with brief vortexing every 2-3 min, then add 150 µL isopropanol and transfer the complete sample to the column.
Gel slice does not dissolve completely	The gel slice was too large for the reagent volume, the 55°C incubation was too short, or mixing during dissolution was insufficient.	Use 300 µL Binding Buffer per 100 mg gel. Incubate for the full 10 min at 55°C and vortex briefly every 2-3 min. Do not add isopropanol or load the column until the agarose is completely dissolved.
Low yield with gel slices larger than 100 mg	Binding Buffer was not increased proportionally when processing more than 100 mg agarose.	For gel slices above 100 mg, increase Binding Buffer proportionally from the 300 µL per 100 mg ratio. After complete dissolution, also keep isopropanol at 150 µL per 100 mg gel.
Adjacent DNA fragments contaminate the purified sample	The band of interest was not fully separated from adjacent fragments before excision, or too much surrounding gel was cut out.	Continue electrophoresis until the band of interest is clearly separated from adjacent contaminating fragments. Excise only the desired band using an ethanol-cleaned scalpel or razor blade.
Wrong DNA band is purified	The target band was not clearly identified or the wrong gel region was excised.	Confirm band separation before excision and cut only the intended DNA band. Use a clean, ethanol-cleaned cutting tool to avoid transferring material from neighboring bands.
Column flow is slow or sample remains on the membrane	Partially dissolved agarose was loaded, the sample volume was too large for one loading step, or centrifugation was not performed at maximum speed.	Load only completely dissolved gel samples. Centrifuge for 30 sec at maximum speed in a tabletop microcentrifuge. If the sample volume is large, load it in successive portions while using the same column.
Wash step gives poor or inconsistent results	Absolute ethanol was not added to the Wash Buffer concentrate before first use, or the specified wash volume was not used.	Add 32 mL absolute ethanol to the 8 mL EcoSpin Wash Buffer concentrate before use. Add 700 µL Wash Buffer to the column and centrifuge for 30 sec at maximum speed.
Residual wash buffer / ethanol in the eluate	The additional empty-column centrifugation after washing was omitted, shortened, or performed below maximum speed.	After the 700 µL wash and 30 sec centrifugation, discard the flowthrough and centrifuge the empty EcoSpin Column for an additional 1 min at maximum speed before transferring it to a clean tube.
Poor downstream performance after purification	Residual Wash Buffer remained on the membrane, or the purified DNA was not fully recovered during elution.	Complete the additional 1 min empty-column spin after washing. Then transfer the column to a clean 1.5 mL tube and perform the elution exactly as specified before using the DNA in downstream applications.
Low DNA concentration in the eluate	Elution Buffer volume was larger than necessary, the 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, incubate for 1 min at room temperature, and centrifuge for 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not contact the membrane adequately, the column was not transferred to a clean tube, or the final centrifugation was incomplete.	Transfer the EcoSpin Column to a clean 1.5 mL microcentrifuge tube, add 30-50 µL Elution Buffer to the membrane center, incubate 1 min, and centrifuge for 30 sec at maximum speed.

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Problem	Most likely cause	Recommended action
Variable recovery between gel samples	Gel mass, Binding Buffer/isopropanol ratios, dissolution time, mixing, wash conditions, or elution conditions differed between samples.	Standardize the workflow by weighing each gel slice, maintaining 300 µL Binding Buffer and 150 µL isopropanol per 100 mg gel, dissolving for 10 min at 55°C with brief mixing, and keeping wash and elution steps identical.
Large amount of agarose surrounding the target band	More gel than necessary was excised around the DNA band, increasing sample mass and reagent requirements.	Excise the smallest practical gel slice containing the desired band. Re-weigh the tube with the excised gel and scale Binding Buffer and isopropanol to the measured gel mass.
Results remain inconsistent despite correct reagent volumes	One or more timing, temperature, mixing, or centrifugation steps differed from the protocol.	Repeat the workflow using 55°C for 10 min during gel dissolution, brief vortexing every 2-3 min, maximum-speed centrifugation for the stated times, the additional 1 min dry spin, and a 1 min room-temperature elution incubation.

Still having trouble? Confirm the Wash Buffer ethanol addition, accurate gel mass, 300 µL Binding Buffer and 150 µL isopropanol per 100 mg gel, complete dissolution at 55°C, 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 gel mass, reagent volumes, dissolution conditions, centrifugation settings, and observed DNA recovery or downstream issue.