

EcoPURE PCR/Gel Purification Kit

Troubleshooting Guide

50 rxns

Cat No: E5003

Use this guide for common problems encountered during PCR product cleanup and agarose gel DNA recovery. The recommendations below follow the product protocol. For best results, verify the Wash Buffer ethanol addition, sample-to-buffer ratios, centrifugation conditions, complete agarose dissolution, and elution steps.

Before You Start - Quick Checks

1. Add 32 mL absolute ethanol to the 8 mL EcoPURE Wash Buffer concentrate before first use.
2. Use a tabletop microcentrifuge capable of >12,000 rpm; perform protocol spin steps at maximum speed and room temperature.
3. For PCR cleanup, standardize each reaction to 50 µL; add 250 µL Binding Buffer and 100 µL isopropanol. For samples >50 µL, scale Binding Buffer proportionally or divide into 50 µL aliquots.
4. When primer-dimers are present, purification may be performed without isopropanol; expect lower recovery of the target DNA fragment.
5. For gel cleanup, use 300 µL Binding Buffer and 150 µL isopropanol per 100 mg agarose. Dissolve at 55°C for 10 min, vortexing briefly every 2-3 min.
6. After washing, centrifuge the empty EcoPURE Column for an additional 1 min at maximum speed to remove residual Wash Buffer.
7. Elute with 30-50 µL EcoPURE Elution Buffer applied to the center of the membrane; incubate 1 min at room temperature and centrifuge 30 sec at maximum speed.

Problem	Most likely cause	Recommended action
Low or no DNA yield after PCR purification	PCR sample volume was not normalized to 50 µL; Binding Buffer/isopropanol amounts were incorrect; sample transfer to the column was incomplete; or centrifugation was not performed at maximum speed.	For each 50 µL PCR product, add 250 µL EcoPURE Binding Buffer and 100 µL isopropanol, mix well, transfer the complete mixture to the column, and centrifuge 30 sec at maximum speed. If the starting PCR volume is larger than 50 µL, increase Binding Buffer proportionally or split the sample into 50 µL aliquots.
Variable yield between PCR reactions	Different reaction volumes were processed with the same reagent volumes, or spin/elution conditions were not kept consistent.	Standardize reactions to 50 µL whenever possible. For smaller samples, adjust total volume to 50 µL. Keep Binding Buffer, isopropanol, wash, centrifugation, and elution conditions identical across samples.
Primer-dimers remain in the purified DNA	The standard PCR cleanup workflow, including isopropanol, may retain more small DNA species than desired.	When primer-dimers are present and their removal is the priority, perform the purification without isopropanol as described in the protocol. Be aware that the yield of the target DNA fragment may decrease.
Target DNA yield decreases when primer-dimer removal is prioritized	Isopropanol was intentionally omitted to improve primer-dimer removal.	This is the trade-off stated in the protocol. If recovery of a defined target band is more important than total PCR cleanup, use the gel-purification workflow to isolate the desired DNA band from agarose.
Poor downstream performance / residual wash components	Residual EcoPURE Wash Buffer remained on the membrane because the additional empty-column centrifugation was omitted or shortened.	After the 700 µL wash and 30 sec centrifugation, discard the flowthrough and centrifuge the empty column for an additional 1 min at maximum speed before transferring it to a clean 1.5 mL tube for elution.
Low or inconsistent elution yield	Elution Buffer was not applied to the center of the membrane, the 1 min room-temperature incubation was skipped, or the elution volume was outside the specified range.	Apply 30-50 µL EcoPURE Elution Buffer directly to the center of the membrane, incubate for 1 min at room temperature, and centrifuge 30 sec at maximum speed.
Sample or liquid remains on the column after centrifugation	Centrifugation was below the specified maximum speed, or a large sample volume was processed without scaling or aliquoting.	Use a tabletop microcentrifuge capable of >12,000 rpm and centrifuge at maximum speed for the specified 30 sec. For PCR volumes >50 µL, increase Binding Buffer proportionally or divide the sample into 50 µL aliquots before loading.
Wash step gives inconsistent results	Absolute ethanol was not added to the Wash Buffer concentrate, or the wash/dry-spin sequence was not followed exactly.	Before first use, add 32 mL absolute ethanol to the 8 mL Wash Buffer concentrate. Wash with 700 µL, centrifuge 30 sec at maximum speed, discard flowthrough, and perform the additional 1 min empty-column spin.

Protocol note: EcoPURE PCR/Gel Purification Kit is stored at 15-25°C and shipped at ambient temperature. Use 96-100% ethanol, 100% isopropanol, sterile 1.5 mL tubes, and a microcentrifuge capable of >12,000 rpm.

EcoPURE Gel Purification - Troubleshooting

Use the gel-specific checks below when recovering DNA from agarose. The protocol is written for 100 mg gel slices and requires complete dissolution before column loading.

Gel Purification - Quick Checks

1. Run the sample on a 0.8-2% agarose gel in 1x TAE or 1x TBE until the band of interest is clearly separated from adjacent fragments.
2. Excise the desired band using an ethanol-cleaned scalpel or razor and determine the gel slice mass by re-weighing the tube.
3. Use 300 µL Binding Buffer per 100 mg gel. Incubate 10 min at 55°C and vortex briefly every 2-3 min until the agarose is completely dissolved.
4. First dissolve the gel slice completely in Binding Buffer at 55°C. After complete dissolution, add the specified isopropanol volume, mix, and load the column.

Problem	Most likely cause	Recommended action
Low or no DNA recovery from the gel slice	Gel mass was not matched to Binding Buffer/isopropanol volumes, the agarose was not completely dissolved, or the sample was not fully transferred to the EcoPURE Column.	Weigh the gel slice accurately. For each 100 mg agarose, add 300 µL Binding Buffer, dissolve completely at 55°C for 10 min with brief vortexing every 2-3 min, then add 150 µL isopropanol and mix well before loading the full sample onto the column.
Agarose slice does not fully dissolve	Incubation at 55°C was too short, or the tube was not mixed during dissolution.	Incubate the gel suspension for the full 10 min at 55°C and vortex briefly every 2-3 min. Do not proceed to isopropanol addition or column loading until the agarose slice is completely dissolved.
Column flow is slow or liquid remains after loading	Partially dissolved agarose was loaded, or the gel mass was increased without a proportional increase in Binding Buffer.	Ensure complete agarose dissolution before loading. For gel slices larger than 100 mg, increase Binding Buffer proportionally from the 300 µL per 100 mg starting ratio, then add isopropanol at 150 µL per 100 mg gel and centrifuge at maximum speed.
Recovery decreases with gel slices larger than 100 mg	The protocol was scaled by gel mass but reagent volumes were not increased proportionally.	Scale Binding Buffer proportionally above 300 µL per 100 mg gel. After complete dissolution, also scale isopropanol at 150 µL per 100 mg gel. Keep all subsequent wash, dry-spin, and elution steps consistent.
Adjacent DNA fragments contaminate the eluate	The target band was not sufficiently separated during electrophoresis, or too much surrounding agarose was excised.	Run the sample in 0.8-2% agarose with 1x TAE or 1x TBE until the desired band is separated from adjacent contaminating fragments. Excise only the desired band with an ethanol-cleaned scalpel or razor.
Desired band is difficult to isolate cleanly	Electrophoresis was stopped before the target band separated from nearby DNA fragments.	Continue electrophoresis until the DNA band of interest is clearly separated. Use the specified 0.8-2% agarose range and 1x TAE or 1x TBE running buffer before excision.
Poor downstream performance after gel purification	Residual Wash Buffer remained on the column because the additional dry spin was omitted, or the column was not transferred to a clean tube before elution.	After the 700 µL wash, centrifuge 30 sec at maximum speed, discard the flowthrough, and centrifuge the empty column for an additional 1 min. Then transfer the column to a clean 1.5 mL microcentrifuge tube before elution.
Low or variable elution after gel purification	Elution Buffer placement, incubation, or centrifugation differed between samples.	Add 30-50 µL EcoPURE Elution Buffer to the center of the membrane, incubate 1 min at room temperature, and centrifuge 30 sec at maximum speed. Apply the same elution conditions to all samples being compared.

Still having trouble? Re-check the Wash Buffer ethanol addition, sample or gel mass, Binding Buffer and isopropanol ratios, centrifuge speed, complete agarose dissolution, 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 sample type and volume, gel mass (if applicable), reagent volumes, centrifugation conditions, and the observed yield or downstream issue.