

EcoSpin Bacterial Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1050

Use this guide for common problems encountered during bacterial genomic DNA purification. For best results, follow the culture input, pellet-resuspension, lysis, Proteinase K digestion, binding, washing, dry-spin, and elution conditions specified 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. Reconstitute lyophilized EcoSpin Proteinase K in 1.1 mL Proteinase K Storage Buffer before use.
3. Use 1 mL overnight bacterial culture per standard preparation; scale all reagents proportionally for higher culture volumes.
4. Harvest bacteria at 6000 rpm for 3 min, remove the supernatant, and completely resuspend the pellet in 200 µL Resuspension Buffer until no clumps remain.
5. For most Gram-positive bacteria, include lysozyme (not provided) to improve lysis of the thick peptidoglycan cell wall.
6. Add 200 µL Lysis Buffer, optional 20 µL RNase A, then 20 µL Proteinase K. Incubate Proteinase K for 10 min at 55°C; extend to 30 min when additional digestion is needed.
7. Add 400 µL Binding Buffer before column loading. Wash with 500 µL Wash Buffer, then 200 µL Wash Buffer followed by a 2 min maximum-speed spin.
8. Elute with 30-50 µL Elution Buffer applied to the membrane center; incubate 5 min at room temperature before a 30 sec maximum-speed centrifugation.

Problem	Most likely cause	Recommended action
Low or no genomic DNA yield	Culture input or reagent volumes were not matched to the standard 1 mL overnight culture protocol; bacterial pellet was not completely resuspended; lysis or Proteinase K digestion was incomplete.	Use 1 mL overnight culture per standard preparation. Resuspend the pellet completely in 200 µL EcoSpin Resuspension Buffer until no clumps remain. Add 200 µL Lysis Buffer and 20 µL Proteinase K, then incubate at 55°C for 10 min. If recovery is low, extend the 55°C incubation up to 30 min as described in the protocol.
Low yield from Gram-positive bacteria	Thick peptidoglycan cell walls were not lysed efficiently.	For most Gram-positive bacteria, include a lysozyme pretreatment because the datasheet specifies that lysozyme is required for effective lysis of thick peptidoglycan walls. Lysozyme is not included in the kit.
Bacterial pellet remains clumped after resuspension	Pellet was not dispersed sufficiently before adding Lysis Buffer.	Resuspend the pellet in 200 µL EcoSpin Resuspension Buffer by vortexing or repeated pipetting until no visible cell clumps remain before proceeding to lysis.
Incomplete lysis / turbid lysate	Cell mass was excessive, pellet resuspension was incomplete, or Proteinase K digestion time was insufficient.	Keep the standard input at 1 mL overnight culture or scale all reagents proportionally for larger inputs. Ensure complete resuspension, mix thoroughly after adding 200 µL Lysis Buffer, and incubate with 20 µL Proteinase K at 55°C for 10 min; extend to 30 min if needed.
RNA contamination in purified DNA	RNase A treatment was omitted in a sample with substantial RNA content.	Use the optional RNase step: add 20 µL EcoSpin RNase A (not provided) after lysis and incubate for 3 min at room temperature before Proteinase K treatment.
Column flow is slow or sample remains on the membrane	Too much culture material was processed without proportional reagent scaling, or the lysate remained particulate because resuspension/lysis was incomplete.	Use the recommended 1 mL culture input, or scale the complete protocol proportionally for larger cultures. Make sure the pellet is fully resuspended and the lysate is thoroughly mixed before adding 400 µL Binding Buffer and loading the column.
Variable DNA yield between preparations	Differences in culture input, pellet resuspension, digestion time, reagent volumes, centrifugation, or elution conditions.	Standardize the workflow: 1 mL overnight culture, 6000 rpm for 3 min for harvesting, 200 µL Resuspension Buffer, 200 µL Lysis Buffer, 20 µL Proteinase K, 400 µL Binding Buffer, and the same wash and elution timing for every preparation.
Poor purity or inhibition in PCR/qPCR/enzymatic reactions	Residual wash components remained on the column or the wash/dry-spin sequence was incomplete.	Confirm that the Wash Buffer concentrate was prepared with 32 mL absolute ethanol. Wash with 500 µL Wash Buffer, centrifuge 30 sec, then add 200 µL Wash Buffer and centrifuge at maximum speed for 2 min to remove residual wash buffer before elution.
Wash Buffer gives poor or inconsistent results	Absolute ethanol was not added to the Wash Buffer concentrate, or the prepared buffer was used inconsistently.	Before first use, add 32 mL absolute ethanol to the 8 mL EcoSpin Wash Buffer concentrate. Mix the prepared Wash Buffer thoroughly and use the protocol-specified 500 µL and 200 µL wash volumes.
Ethanol / wash-buffer carryover in the eluate	The final 2 min centrifugation after the 200 µL wash was omitted, shortened, or not performed at maximum speed.	After the 500 µL wash, add 200 µL Wash Buffer and centrifuge the EcoSpin Column at maximum speed for the full 2 min. Transfer the column to a clean 1.5 mL tube before adding Elution Buffer.

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Problem	Most likely cause	Recommended action
Low DNA concentration in the eluate	Elution volume was too high for the desired concentration, Elution Buffer was not applied to the membrane center, or the 5 min incubation was skipped.	Elute with 30-50 µL EcoSpin Elution Buffer applied directly to the center of the membrane. Incubate for 5 min at room temperature before centrifuging for 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not contact the membrane adequately, or centrifugation conditions were insufficient.	Apply 30-50 µL Elution Buffer to the membrane center, incubate 5 min at room temperature, and centrifuge 30 sec at maximum speed. Confirm that the column is placed in a clean 1.5 mL microcentrifuge tube before elution.
Proteinase K performance is reduced	Lyophilized Proteinase K was not reconstituted correctly or the solution was stored outside the specified conditions.	Reconstitute Proteinase K in 1.1 mL Proteinase K Storage Buffer. The reconstituted solution is stable for 1 year at 4°C; for long-term storage beyond 1 year, store at -20°C.
Higher culture volume gives unexpectedly low recovery	The amount of starting culture was increased without proportional scaling of all reagents and processing steps.	The standard protocol is designed for 1 mL overnight bacterial culture. If a higher culture volume is used, scale the amounts of reagents throughout the entire protocol proportionally rather than increasing only one or two reagents.
DNA yield improves only after prolonged digestion	The bacterial sample requires more complete Proteinase K digestion than achieved during the standard 10 min incubation.	The protocol explicitly allows extending the Proteinase K incubation at 55°C from 10 min up to 30 min to help increase DNA yield. Keep all other reagent volumes unchanged for the standard 1 mL culture input.
Cross-sample variability or possible contamination	Inconsistent sample handling, incomplete removal of culture supernatant, or transfer between tubes/columns was not performed cleanly.	Harvest each culture under the same conditions, remove the supernatant carefully, use clean tubes and tips, and transfer the complete sample to the EcoSpin Column while avoiding sample mix-up between preparations.

Still having trouble? Confirm Wash Buffer ethanol addition, Proteinase K reconstitution/storage, the 1 mL culture input, complete pellet resuspension, Gram-positive lysozyme pretreatment when appropriate, 55°C Proteinase K digestion, the 2 min final wash spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the bacterial strain, culture volume, lysis pretreatment, incubation times, centrifugation conditions, and observed DNA yield or purity.