

EcoSpin Cell Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1055

Use this guide for common problems encountered during genomic DNA purification from cultured cells. For best results, follow the specified cell-input range, complete pellet resuspension, RNase A and Proteinase K treatments, binding, wash, final dry-spin, and elution conditions in the product protocol.

Before You Start - Quick Checks

1. Add 8.8 mL absolute ethanol to EcoSpin Wash Buffer 1 and 32 mL absolute ethanol to the 8 mL Wash Buffer 2 concentrate before first use.
2. Reconstitute lyophilized Proteinase K in 1.1 mL Proteinase K Storage Buffer and RNase A in 1.1 mL RNase Reconstitution Buffer.
3. Use 10^4 - 10^6 cultured cells per standard preparation. For more cells, scale the amounts of reagents used in the entire protocol proportionally.
4. Completely resuspend the cell pellet in 200 μ L Resuspension Buffer until no cell clumps remain, then add 200 μ L Lysis Buffer and mix thoroughly.
5. Add 20 μ L RNase A and incubate 3 min at room temperature. Then add 20 μ L Proteinase K and incubate 10 min at 55°C; extend to 30 min when additional digestion is needed.
6. Add 400 μ L Binding Buffer before column loading and centrifuge the loaded column for 1 min at maximum speed at room temperature.
7. Wash sequentially with 400 μ L Wash Buffer 1, 500 μ L Wash Buffer 2, then 200 μ L Wash Buffer 2 followed by a 2 min maximum-speed spin.
8. Elute with 30-50 μ L 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	Cell input or reagent volumes were outside the standard 10^4 - 10^6 cell workflow; the pellet was not completely resuspended; or lysis/Proteinase K digestion was incomplete.	Use 10^4 - 10^6 cells per standard preparation. Completely resuspend the pellet in 200 μ L EcoSpin Resuspension Buffer, then use 200 μ L Lysis Buffer, 20 μ L RNase A, 20 μ L Proteinase K, and 400 μ L Binding Buffer in the stated order. Complete all incubation and centrifugation steps before elution.
Low yield when processing more than 10^6 cells	A cell input above the validated range was processed without increasing reagent volumes.	For more than 10^6 cells, scale up the amounts of reagents used in the entire protocol proportionally, as specified in the datasheet.
Cell pellet does not resuspend completely / visible clumps remain	The pellet was not dispersed sufficiently in Resuspension Buffer before lysis.	Resuspend the cell pellet in 200 μ L EcoSpin Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain before adding Lysis Buffer.
Incomplete cell lysis	Lysis Buffer was not mixed thoroughly or Proteinase K digestion was too short.	After complete resuspension, add 200 μ L EcoSpin Lysis Buffer and mix thoroughly. Add 20 μ L Proteinase K and incubate at 55°C for 10 min. If needed, extend the Proteinase K incubation to 30 min as described in the protocol.
RNA contamination in purified DNA	RNase A was omitted, not reconstituted correctly, or the 3 min room-temperature incubation was shortened.	Add 20 μ L reconstituted EcoSpin RNase A after Lysis Buffer addition, mix well, and incubate for 3 min at room temperature before Proteinase K treatment.
Column flow is slow or sample remains on the membrane	The sample input exceeded the standard range, cell clumps remained, or lysis/digestion was incomplete before loading.	Keep the standard input within 10^4 - 10^6 cells or scale all reagents proportionally for higher input. Ensure the pellet is fully resuspended and the lysate is thoroughly mixed before adding 400 μ L Binding Buffer and loading the column. Centrifuge for 1 min at maximum speed.
Variable yield between cell preparations	Differences in cell input, pellet resuspension, incubation time, reagent volumes, centrifugation, wash steps, or elution conditions.	Standardize cell number and follow the same reagent volumes and timing for each sample. Use complete pellet resuspension, 3 min RNase A treatment, 10 min Proteinase K digestion at 55°C, the specified wash sequence, and 30-50 μ L elution.
Poor purity or inhibition in downstream PCR/qPCR/Southern blotting/enzymatic reactions	Wash Buffer preparation was incorrect or residual wash solution remained on the membrane because the final wash/dry-spin sequence was incomplete.	Confirm ethanol was added to both Wash Buffers. Wash with 400 μ L Wash Buffer 1, then 500 μ L Wash Buffer 2, followed by 200 μ L Wash Buffer 2 and a full 2 min centrifugation at maximum speed to remove residual wash buffer.
Wash Buffer 1 gives poor or inconsistent results	Absolute ethanol was not added to Wash Buffer 1 concentrate before first use.	Add 8.8 mL absolute ethanol to EcoSpin Wash Buffer 1 concentrate before use. Mix thoroughly and use 400 μ L per column, followed by a 1 min maximum-speed centrifugation at room temperature.
Wash Buffer 2 gives poor or inconsistent results	Absolute ethanol was not added to Wash Buffer 2 concentrate before first use.	Add 32 mL absolute ethanol to the 8 mL EcoSpin Wash Buffer 2 concentrate. Use 500 μ L for the first Wash Buffer 2 step, then 200 μ L followed by a 2 min maximum-speed centrifugation.

Table continues on next page.

EcoSpin Cell Genomic DNA Kit Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Ethanol / wash-buffer carryover in the eluate	The final 2 min centrifugation after the 200 µL Wash Buffer 2 step was omitted, shortened, or not performed at maximum speed.	After the 500 µL Wash Buffer 2 wash, add 200 µL Wash Buffer 2 and centrifuge at maximum speed for the full 2 min. Transfer the column to a clean 1.5 mL microcentrifuge tube before elution.
Low DNA concentration in the eluate	The elution volume was larger than needed, Elution Buffer was not applied to the center of the membrane, or the 5 min incubation was skipped.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the column membrane, incubate for 5 min at room temperature, then centrifuge for 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not adequately contact the membrane or the elution incubation/centrifugation step was incomplete.	Transfer the column to a clean 1.5 mL tube, add 30-50 µL Elution Buffer to the membrane center, incubate for 5 min at room temperature, and centrifuge for 30 sec at maximum speed.
Proteinase K performance is reduced	Lyophilized Proteinase K was not reconstituted in the specified volume or the reconstituted solution was not stored according to the datasheet.	Reconstitute Proteinase K in 1.1 mL Proteinase K Storage Buffer. The solution is stable for 1 year at 4°C; for storage longer than 1 year, store it at -20°C.
RNase A performance is reduced	Lyophilized RNase A was not reconstituted in the specified buffer/volume or the prepared solution was stored outside the stated conditions.	Reconstitute RNase A in 1.1 mL RNase Reconstitution Buffer. The solution is stable for 1 year at 4°C; for storage longer than 1 year, store it at -20°C.
Results remain inconsistent despite correct reagent volumes	One or more incubation or centrifugation steps differed from the protocol, including the binding spin, wash spins, final 2 min spin, or 5 min elution incubation.	Repeat the workflow using the specified room-temperature and maximum-speed centrifugation conditions. Keep the 1 min column-binding spin, 1 min Wash Buffer 1 spin, 30 sec Wash Buffer 2 spin, final 2 min spin, and 5 min elution incubation consistent across samples.

Still having trouble? Confirm both Wash Buffer ethanol additions, Proteinase K and RNase A reconstitution/storage, the 10^4 - 10^6 cell input range, complete pellet resuspension, 55°C Proteinase K digestion, the full wash sequence with the final 2 min spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the cell number, reagent volumes, incubation times, centrifugation conditions, and observed DNA yield or downstream performance.