

EcoSpin Yeast Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1060

Use this guide for common problems encountered during genomic DNA purification from yeast cells. For best results, follow the specified cell-input limit, complete pellet resuspension, lyticase treatment, RNase A and Proteinase K incubations, column washing, 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 up to 10^8 yeast cells per standard preparation; if more cells are processed, scale the reagents used in the entire protocol proportionally.
4. Resuspend the yeast pellet completely in 200 μ L Resuspension Buffer until no cell clumps remain.
5. Add 10 μ L lyticase (0.5 mg/mL; not provided) and incubate for 30 min at 37°C before chemical lysis.
6. Add 200 μ L Lysis Buffer, 20 μ L RNase A for 3 min at room temperature, then 20 μ L Proteinase K for 10 min at 55°C. Incubation may be extended to 30 min to improve yield.
7. Add 400 μ L Binding Buffer, load the column, and follow the specified Wash Buffer 1 / Wash Buffer 2 sequence including the final 2 min maximum-speed spin.
8. Elute with 30-50 μ L Elution Buffer applied to the membrane center; incubate for 5 min at room temperature before a 30 sec maximum-speed centrifugation.

Problem	Most likely cause	Recommended action
Low or no genomic DNA yield	Yeast input exceeded the standard range, the pellet was incompletely resuspended, lyticase treatment was insufficient, Proteinase K digestion was incomplete, or the binding/wash/elution sequence was not followed as specified.	Use up to 10^8 yeast cells per standard preparation. Resuspend completely in 200 μ L Resuspension Buffer, treat with 10 μ L lyticase (0.5 mg/mL) for 30 min at 37°C, then follow the Lysis Buffer, RNase A, Proteinase K, Binding Buffer, wash, and elution steps exactly as described.
Poor yield when processing more than 10^8 cells	More cells were processed using the standard reagent volumes, reducing effective lysis and column performance.	The standard procedure is specified for up to 10^8 yeast cells. If more cells are used, scale up the amounts of reagents used in the entire protocol proportionally.
Cell pellet is difficult to resuspend / clumps remain	The pellet was not fully dispersed in Resuspension Buffer before enzymatic lysis.	Resuspend the pellet in 200 μ L EcoSpin Resuspension Buffer by vortexing or pipetting up and down until no visible cell clumps remain before adding lyticase.
Incomplete yeast cell lysis	Lyticase was omitted, used at the wrong concentration/volume, or the 30 min incubation at 37°C was shortened.	Add 10 μ L lyticase at 0.5 mg/mL to the resuspended cells and incubate for the full 30 min at 37°C before adding EcoSpin Lysis Buffer.
Yield remains low despite lyticase treatment	Proteinase K digestion after chemical lysis was insufficient.	After adding 200 μ L Lysis Buffer and completing RNase A treatment, add 20 μ L Proteinase K and incubate for 10 min at 55°C. The protocol notes that extending this incubation to 30 min can help increase yield.
RNA contamination in purified DNA	RNase A was omitted, incompletely reconstituted, or the 3 min room-temperature incubation was shortened.	Add 20 μ L reconstituted EcoSpin RNase A after the 200 μ L Lysis Buffer step and incubate for 3 min at room temperature before adding Proteinase K.
DNA does not bind efficiently to the column	The 400 μ L Binding Buffer step was omitted, measured incorrectly, or the lysate was not mixed sufficiently before column loading.	After Proteinase K digestion, add 400 μ L EcoSpin Binding Buffer and mix well before transferring the sample to the EcoSpin Column.
Sample remains on the column after the binding spin	The sample was not centrifuged at maximum speed for the full 1 min or the lysate remained unusually viscous/incompletely lysed.	Ensure complete enzymatic lysis before loading and centrifuge the loaded column for 1 min at maximum speed at room temperature, as specified.
Column flow is slow or inconsistent	Residual cell aggregates or incompletely lysed material reached the column, increasing resistance through the membrane.	Confirm complete pellet resuspension and completion of both lyticase and Proteinase K incubations before loading the column. Do not proceed while visible clumps remain.
Poor purity / inhibition in PCR, qPCR, Southern blotting, or enzymatic reactions	Wash Buffers were not prepared with ethanol or residual wash solution remained on the membrane after the final wash step.	Verify that 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 maximum-speed centrifugation.
Wash Buffer 1 gives variable results	The required 8.8 mL 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. Use 400 μ L per column and centrifuge for 1 min at maximum speed at room temperature.

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EcoSpin Yeast Genomic DNA Kit Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Wash Buffer 2 gives variable results	The required 32 mL absolute ethanol was not added to the 8 mL Wash Buffer 2 concentrate before first use.	Add 32 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate. Use 500 µL for the first Wash Buffer 2 step, then 200 µL followed by a 2 min maximum-speed centrifugation.
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. Then transfer the column to a clean 1.5 mL tube before elution.
Low DNA concentration in the eluate	Elution Buffer was not applied to the center of the membrane, the 5 min room-temperature incubation was skipped, or an unnecessarily large elution volume was used.	Apply 30-50 µL EcoSpin Elution Buffer directly to the membrane center, 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 fully contact the membrane or the final 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 5 min, 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 prepared solution was stored outside the stated conditions.	Reconstitute Proteinase K in 1.1 mL Proteinase K Storage Buffer. The prepared solution is stable for 1 year at 4°C; for storage longer than 1 year, store 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 prepared solution is stable for 1 year at 4°C; for storage longer than 1 year, store at -20°C.
Variable results between yeast preparations	Starting cell number, pellet resuspension, lyticase treatment, Proteinase K digestion, reagent preparation, centrifugation, or elution conditions varied between samples.	Standardize the workflow: use no more than 10 ⁸ cells, fully resuspend the pellet, complete the 30 min lyticase step and 55°C Proteinase K digestion, verify Wash Buffer ethanol additions, use the full 2 min final wash spin, and keep elution conditions consistent.

Still having trouble? Confirm the yeast input does not exceed 10⁸ cells without proportional scaling, the pellet is fully resuspended, lyticase was used at 10 µL of 0.5 mg/mL for 30 min at 37°C, RNase A and Proteinase K incubations were completed, both Wash Buffers were prepared with ethanol, the final 2 min wash spin was performed at maximum speed, and elution used 30-50 µL buffer applied to the membrane center. If the problem persists, contact EcoTech Biotechnology technical support with the cell input, lyticase details, incubation times, reagent volumes, centrifugation conditions, and observed DNA yield or downstream performance.