

EcoSpin Tissue Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1070

Use this guide for common problems encountered during genomic DNA purification from fresh or frozen tissue samples and rodent tails. For best results, follow the specified starting-material limits, sample disruption, 55°C Proteinase K digestion, RNase A treatment, 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 up to 25 mg fresh/frozen tissue, 0.6-1.2 cm mouse tail, or 0.6 cm rat tail per standard preparation; scale the entire protocol proportionally when using more material.
4. Cut tissue or rodent tail into small pieces. If available, grind the sample in liquid nitrogen to reduce lysis duration.
5. Add 200 µL Resuspension Buffer, 200 µL Tissue Lysis Buffer, and 20 µL Proteinase K; incubate at 55°C until complete lysis.
6. The datasheet notes typical lysis times of 1-3 h for tissues and 6-8 h for rodent tails; vortexing every 15 min may shorten lysis and improve yield.
7. After lysis, add 20 µL RNase A for 3 min at room temperature, then 400 µL Binding Buffer and 200 µL absolute ethanol before column loading.
8. Elute with 30-50 µL Elution Buffer applied to the membrane center; incubate 5 min at room temperature before the final 30 sec centrifugation.

Problem	Most likely cause	Recommended action
Low or no genomic DNA yield	Starting material exceeded the standard range; tissue or tail was not reduced to small pieces; lysis was incomplete; or the binding, wash, or elution steps were not completed as specified.	Use up to 25 mg fresh/frozen tissue, 0.6-1.2 cm mouse tail, or 0.6 cm rat tail per standard preparation. Cut the sample into small pieces or grind in liquid nitrogen, complete Proteinase K digestion at 55°C, and follow the binding, wash, and elution steps exactly.
Yield is lower than the expected range	Tissue disruption or Proteinase K digestion was incomplete, especially with dense tissue or rodent tail material.	Cut the material into small pieces and, when available, grind in liquid nitrogen to shorten lysis. Incubate at 55°C until the sample is completely lysed. The protocol notes typical lysis times of 1-3 h for tissues and 6-8 h for rodent tails.
Poor results with more than 25 mg tissue or longer tail pieces	More starting material was processed with the standard reagent volumes.	When processing more tissue than the stated input, scale the amounts of reagents used in the entire protocol proportionally.
Incomplete lysis / visible tissue remains	The sample pieces were too large, tissue was not ground, or Proteinase K incubation was stopped before complete lysis.	Cut tissue or rodent tail into small pieces. If available, grind in liquid nitrogen. Add 200 µL Resuspension Buffer, 200 µL Tissue Lysis Buffer, and 20 µL Proteinase K, then incubate at 55°C until complete lysis.
Rodent tail lysis is very slow	Rodent tail material generally requires a longer digestion than soft tissue.	Continue the 55°C incubation until complete lysis. The datasheet states that rodent tails commonly require 6-8 h. Vortexing every 15 min may decrease lysis duration and increase DNA yield.
Tissue lysis takes longer than expected	The tissue was not sufficiently cut or disrupted before adding lysis reagents.	Reduce the sample to small pieces before lysis, or grind in liquid nitrogen if available. During the 55°C incubation, vortex every 15 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 µL reconstituted EcoSpin RNase A after lysis and incubate for 3 min at room temperature before adding Binding Buffer and ethanol.
DNA does not bind efficiently to the column	Binding Buffer and/or absolute ethanol was omitted, added at an incorrect volume, or not mixed thoroughly.	After RNase A treatment, add 400 µL EcoSpin Binding Buffer, then 200 µL absolute ethanol, and mix well before loading the EcoSpin Column.
Column flow is slow or the membrane clogs	The sample was not completely lysed or excessive starting material was loaded without proportional reagent scaling.	Do not proceed to binding until the tissue is completely lysed. Keep the starting material within the stated range or proportionally scale the entire protocol when using more sample.
Sample remains on the column after the binding spin	The binding mixture was too viscous because of incomplete lysis or the centrifugation was not performed at maximum speed for the full 1 min.	Confirm complete tissue lysis and thorough mixing after Binding Buffer and ethanol addition. Centrifuge the loaded column for 1 min at maximum speed at room temperature.
Poor purity / inhibition in downstream PCR, qPCR, Southern blotting, or enzymatic reactions	Wash buffers were not prepared correctly or residual wash solution remained on the membrane because the final wash sequence was incomplete.	Confirm ethanol was added to both Wash Buffers. Wash with 400 µL Wash Buffer 1, then 500 µL Wash Buffer 2, followed by 200 µL Wash Buffer 2 and a full 2 min maximum-speed spin.

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Problem	Most likely cause	Recommended action
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. Use 400 µL per column and centrifuge for 1 min at maximum speed 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 µL for the first Wash Buffer 2 step, then 200 µL followed by a 2 min maximum-speed spin.
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.	Perform the complete final Wash Buffer 2 step and centrifuge for the full 2 min at maximum speed. 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 incubation was skipped, or a larger elution volume than needed was used.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the membrane, incubate for 5 min at room temperature, and centrifuge for 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not adequately 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 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 solution is stable for 1 year at 4°C; for storage longer than 1 year, store at -20°C.
Variable results between tissue or tail samples	Differences in starting amount, sample disruption, lysis completion, reagent preparation, wash steps, or elution conditions.	Standardize the workflow: keep input within the stated limits, use consistent sample cutting/grinding, incubate until complete lysis, use the specified binding and wash volumes, complete the 2 min final wash spin, and keep the 30-50 µL elution conditions consistent.

Still having trouble? Confirm the starting-material limit, adequate cutting or liquid-nitrogen grinding, complete Proteinase K digestion at 55°C, RNase A treatment, correct Binding Buffer and ethanol volumes, ethanol addition to both Wash Buffers, the final 2 min Wash Buffer 2 spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the sample type and amount, lysis time, reagent volumes, centrifugation conditions, and observed DNA yield or downstream performance.