

EcoSpin FFPE Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E3095

Use this guide for common problems encountered during genomic DNA purification from formalin-fixed, paraffin-embedded (FFPE) tissue. For best results, follow the specified section input, deparaffinization, complete tissue lysis, 90°C incubation, binding, wash, 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 40 mL absolute ethanol to Wash Buffer 2 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 5 freshly cut FFPE sections, each up to 20 µm thick, from the interior of the block; total recommended starting surface area is up to 250 mm².
4. Remove paraffin with 1 mL xylene at 50°C for 5 min, then perform two 1 mL 96-100% ethanol washes and air dry the pellet for about 10 min.
5. Add 100 µL Pre-Lysis Buffer, 100 µL FFPE Lysis Buffer, and 20 µL Proteinase K. Incubate at 55°C for at least 3 h or until completely lysed, with frequent vortexing.
6. Incubate at 90°C for 1 h with frequent vortexing, cool to room temperature, then add 20 µL RNase A and incubate 3 min.
7. Add 400 µL Binding Buffer followed by 200 µL absolute ethanol, then load the column and follow the complete wash sequence including the final 2 min spin.
8. Elute with 30-50 µL Elution Buffer applied to the membrane center; incubate 5 min at room temperature and centrifuge for 1 min at maximum speed.

Problem	Most likely cause	Recommended action
Low or no genomic DNA yield	Too much starting material was used, deparaffinization was incomplete, tissue lysis was incomplete, or the binding/elution steps were not performed as specified.	Use up to 5 freshly cut FFPE sections of up to 20 µm thickness and a total surface area up to 250 mm ² per standard preparation. Remove paraffin completely, complete the 55°C lysis and 90°C incubation, then follow the Binding Buffer, ethanol, column, wash, and elution steps exactly.
Paraffin remains visible after deparaffinization	Excess paraffin was not removed before xylene treatment, or the xylene incubation/mixing step was incomplete.	Remove excess paraffin before starting. Add 1 mL xylene, incubate at 50°C for 5 min, vortex, centrifuge at maximum speed for 2 min, and carefully remove the xylene completely before proceeding.
Residual xylene remains in the sample	The xylene supernatant was not completely removed after centrifugation.	After the 2 min maximum-speed spin, carefully remove the xylene completely. Then proceed with the two sequential 96-100% ethanol wash steps described in the protocol.
Residual ethanol remains before lysis	The pellet was not air dried for the full period after the repeated ethanol washes.	After repeating the 1 mL ethanol wash and centrifugation, air dry the pellet for about 10 min at room temperature to completely remove residual ethanol before adding Pre-Lysis Buffer.
Tissue is not completely lysed	Proteinase K digestion was too short, mixing during incubation was insufficient, or the tissue amount exceeded the standard input.	Add 100 µL Pre-Lysis Buffer, 100 µL FFPE Lysis Buffer, and 20 µL Proteinase K. Incubate at 55°C for at least 3 h or until the tissue is completely lysed, with frequent vortexing. For larger inputs, scale all reagents proportionally.
Lysate remains heterogeneous after the 55°C step	The sample has not reached complete lysis or was not mixed frequently during incubation.	Continue the 55°C incubation until complete lysis is achieved and vortex frequently during the incubation, as specified in the protocol, before proceeding to the 90°C step.
Inconsistent results after the 90°C incubation	The 90°C incubation was shortened or the sample was not mixed frequently.	After complete 55°C lysis, incubate the sample at 90°C for the full 1 h with frequent vortexing. Cool the sample to room temperature before adding RNase A.
RNA contamination in purified DNA	RNase A was omitted, incorrectly reconstituted, or the 3 min room-temperature incubation was shortened.	After the 90°C step and cooling to room temperature, add 20 µL EcoSpin RNase A and incubate for 3 min at room temperature before adding Binding Buffer.
Column flow is slow or sample remains on the membrane	The starting material exceeded the recommended amount, tissue lysis was incomplete, or the lysate was not mixed thoroughly after Binding Buffer and ethanol addition.	Stay within the recommended FFPE input or scale the complete protocol proportionally. Ensure complete tissue lysis, add 400 µL Binding Buffer and 200 µL absolute ethanol, vortex for 10 sec, then load the column and centrifuge for 30 sec at maximum speed.
Poor purity / residual wash components in eluate	Wash Buffers were not prepared with ethanol or the final 2 min Wash Buffer 2 spin was incomplete.	Confirm that 8.8 mL absolute ethanol was added to Wash Buffer 1 and 40 mL absolute ethanol to Wash Buffer 2. Follow the 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, then 200 µL Wash Buffer 2 sequence, finishing with a 2 min maximum-speed spin.
Wash Buffer 1 gives inconsistent results	Absolute ethanol was not added to Wash Buffer 1 concentrate before use.	Add 8.8 mL absolute ethanol to EcoSpin Wash Buffer 1 before use. Apply 400 µL to the column and centrifuge for 30 sec at maximum speed at room temperature.

Table continues on next page.

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Problem	Most likely cause	Recommended action
Wash Buffer 2 gives inconsistent results	Absolute ethanol was not added to Wash Buffer 2 concentrate before use.	Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before use. Use 500 µL for the first Wash Buffer 2 step, then 200 µL followed by the full 2 min maximum-speed centrifugation.
Low DNA concentration in the eluate	Elution Buffer was not applied to the membrane center, the 5 min incubation was skipped, or the elution volume was larger than the specified range.	Transfer the column to a clean DNase-free 1.5 mL tube. Add 30-50 µL EcoSpin Elution Buffer to the center of the membrane, incubate for 5 min at room temperature, then centrifuge for 1 min at maximum speed.
No or incomplete eluate collection	The final centrifugation was shortened or complete collection of the eluate was not confirmed.	After the 5 min elution incubation, centrifuge for the full 1 min at maximum speed at room temperature and confirm that the eluate has been completely collected in the receiving tube.
Proteinase K performance is reduced	Lyophilized Proteinase K was not reconstituted in the specified volume or the prepared solution was not stored as directed.	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.
Variable results between FFPE preparations	Differences in section number/thickness, paraffin removal, lysis completion, incubation times, wash preparation, or elution conditions.	Standardize starting material and follow the same xylene/ethanol deparaffinization, 55°C and 90°C incubations, reagent volumes, wash sequence, and 30-50 µL elution conditions for all samples being compared.

Still having trouble? Re-check the FFPE section input, complete xylene removal, both ethanol deparaffinization washes, the 10 min air-dry step, complete 55°C Proteinase K lysis, the full 90°C incubation, Wash Buffer ethanol additions, final 2 min wash spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the section number/thickness, approximate tissue area, incubation times, reagent volumes, centrifugation conditions, and observed DNA yield or downstream performance.