

EcoSpin FFPE Total RNA Kit

Troubleshooting Guide

50 rxns

Cat No: E2095

Use this guide for common problems encountered during total RNA purification from formalin-fixed, paraffin-embedded (FFPE) tissue. For best results, follow the specified FFPE section input, complete deparaffinization, 55°C and 80°C incubations, clarification, binding, wash, and elution conditions in the product protocol.

Before You Start - Quick Checks

1. Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before first use.
2. Reconstitute lyophilized Proteinase K in 1.1 mL Proteinase K Storage Buffer. Store the solution at 4°C for up to 1 year or at -20°C for longer-term storage.
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 15 min with frequent vortexing; extending this time does not increase yield.
6. Incubate at 80°C for 15 min with frequent vortexing, centrifuge at maximum speed for 1 min, and transfer only the supernatant to a new RNase-free tube.
7. Add 400 µL Binding Buffer and 600 µL absolute 96-100% ethanol, mix for 10 sec, then load the column and complete the full wash sequence including the final 2 min spin.
8. Elute with 50-100 µL Elution Buffer applied to the membrane center; incubate 1 min at room temperature and centrifuge for 30 sec at maximum speed.

Problem	Most likely cause	Recommended action
Low or no total RNA yield	Starting material exceeded the recommended FFPE input, paraffin removal was incomplete, tissue lysis was incomplete, or binding/elution conditions were not followed as specified.	Use up to 5 freshly cut FFPE sections of up to 20 µm thickness and a total recommended surface area up to 250 mm². Complete xylene/ethanol deparaffinization, 55°C Proteinase K lysis, 80°C incubation, clarification, binding, wash, and elution steps exactly as described.
Paraffin remains visible after deparaffinization	Excess paraffin was not removed before xylene treatment, or xylene incubation/mixing was incomplete.	Remove excess paraffin first. 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 centrifugation, carefully remove the xylene completely. Then perform the two sequential 1 mL 96-100% ethanol washes 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 the second 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 adequately lysed	Proteinase K digestion or mixing during the 55°C incubation was insufficient.	Add 100 µL Pre-Lysis Buffer, 100 µL FFPE Lysis Buffer, and 20 µL Proteinase K. Incubate at 55°C for 15 min with frequent vortexing. Follow the stated time; the protocol notes that extending this incubation does not increase yield.
Yield does not improve after extending Proteinase K incubation	The 55°C Proteinase K incubation was extended beyond the protocol in an attempt to increase recovery.	Use the specified 15 min incubation at 55°C with frequent vortexing. The product protocol specifically states that extending this incubation time will not increase the yield.
Inconsistent results after the 80°C step	The 80°C incubation was shortened or the sample was not mixed frequently.	After the 55°C lysis, incubate at 80°C for the full 15 min with frequent vortexing before clarification.
Cloudy lysate / particulate material after heating	The clarification spin was omitted or the pellet was transferred with the supernatant.	After the 80°C incubation, centrifuge at maximum speed for 1 min. Transfer only the supernatant to a new RNase-free microcentrifuge tube and discard the pellet.
Column flow is slow or sample remains on the membrane	Particulate material was carried over, starting material exceeded the recommended input, or the binding mixture was not prepared correctly.	Clarify the lysate and transfer only the supernatant. Stay within the recommended input or scale the complete protocol proportionally. Add 400 µL Binding Buffer followed by 600 µL absolute ethanol, mix by vortexing for 10 sec, and load the column in repeated portions if necessary.
Poor RNA binding / unexpectedly low recovery	Binding Buffer or absolute ethanol was omitted, or the ethanol volume was incorrect.	To the clarified supernatant, add 400 µL EcoSpin Binding Buffer and then 600 µL absolute 96-100% ethanol. Mix well by vortexing for 10 sec before loading the column.
Genomic DNA contamination affects sensitive downstream applications	Residual genomic DNA remains after the standard isolation workflow.	The kit generally yields RNA with minimal genomic DNA contamination. For maximum removal of residual DNA when required, apply the optional On-Column DNA Removal step described by EcoTech.

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 the Wash Buffer 2 concentrate before first use.	Add 40 mL absolute ethanol to the EcoSpin Wash Buffer 2 concentrate before use. Then follow the 500 µL wash and final 200 µL Wash Buffer 2 spin sequence.
Residual wash components / ethanol carryover in eluate	The final Wash Buffer 2 step or the full 2 min maximum-speed centrifugation was omitted or shortened.	After the 500 µL Wash Buffer 2 wash, add 200 µL Wash Buffer 2 and centrifuge at maximum speed for the full 2 min to completely remove residual wash buffer before elution.
Low RNA concentration in eluate	Elution volume was larger than necessary, Elution Buffer was not applied to the membrane center, or the 1 min incubation was skipped.	Transfer the column to a clean RNase-free 1.5 mL tube. Add 50-100 µL EcoSpin Elution Buffer to the center of the membrane, incubate for 1 min at room temperature, and centrifuge for 30 sec at maximum speed.
No or incomplete eluate collection	The final centrifugation was shortened or the receiving tube/column was not positioned correctly.	After the 1 min elution incubation, centrifuge for the full 30 sec at maximum speed at room temperature and confirm that eluate has collected in the RNase-free receiving tube.
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 it at -20°C.
Variable results between FFPE preparations	Differences in section number/thickness, tissue area, completeness of paraffin removal, incubation times, clarification, wash preparation, or elution conditions.	Standardize starting material and follow the same xylene/ethanol deparaffinization, 55°C and 80°C incubations, clarification, Binding Buffer/ethanol volumes, wash sequence, and 50-100 µL elution conditions for all samples being compared.
Poor downstream performance after isolation	Residual wash components, residual genomic DNA in a sensitive assay, or inconsistent handling of the RNA after purification.	Confirm the complete final 2 min wash spin and use optional On-Column DNA Removal when residual DNA may affect the application. Store purified RNA at -20°C for a few days or -80°C for long-term storage as specified.

Still having trouble? Re-check the FFPE section input, complete xylene removal, both ethanol deparaffinization washes, the 10 min air-dry step, the specified 15 min Proteinase K lysis at 55°C, the full 15 min incubation at 80°C, clarification before binding, Wash Buffer 2 ethanol addition, the final 2 min wash spin, and 50-100 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 RNA yield or downstream issue.