

EcoSpin Blood Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: EcoBGD-50x

Use this guide for common problems encountered during genomic DNA purification from whole blood. For best results, follow the specified 200 µL blood input, lysis, RNase A and Proteinase K 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 200 µL whole blood per standard preparation. For higher blood volumes, use Red Blood Cell Lysis Buffer (10x, Cat No: RBCLB-10x) to recover white blood cells from up to 9 mL blood before continuing.
4. Add 200 µL Lysis Buffer, then 20 µL RNase A and incubate 3 min at room temperature. Add 20 µL Proteinase K and incubate 10 min at 55°C; extend to 30 min when additional digestion is needed.
5. For parasite DNA isolation, extend the Proteinase K incubation at 55°C from approximately 3 hours to overnight.
6. Add 400 µL Binding Buffer before column loading and centrifuge for 1 min at maximum speed at room temperature.
7. Wash sequentially with 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, then 200 µL Wash Buffer 2 followed by a 2 min maximum-speed spin.
8. Elute with 30-50 µL 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	Blood input or reagent volumes were not matched to the standard 200 µL whole-blood protocol; lysis or Proteinase K digestion was incomplete; or centrifugation conditions were not followed.	Use 200 µL whole blood per standard preparation. Add 200 µL EcoSpin Lysis Buffer, 20 µL RNase A, 20 µL Proteinase K, and 400 µL Binding Buffer in the specified order. Incubate with Proteinase K at 55°C for 10 min; if recovery is low, extend the incubation up to 30 min as described in the protocol.
Low yield when processing more than 200 µL whole blood	The standard column protocol was used directly with a blood volume above 200 µL.	For blood volumes higher than 200 µL, use EcoTech Red Blood Cell Lysis Buffer (10x, Cat No: RBCLB-10x) to isolate white blood cells from up to 9 mL whole blood, then continue the genomic DNA workflow with the recovered cells.
Low DNA recovery from parasite-containing blood samples	The standard 10-30 min Proteinase K digestion was insufficient for the intended parasite DNA recovery.	For parasite DNA isolation, extend the 55°C Proteinase K incubation from approximately 3 hours to overnight, as specified in the datasheet, before proceeding to Binding Buffer addition and column loading.
Incomplete lysis / viscous sample before column loading	Lysis Buffer was not mixed thoroughly with the blood sample or Proteinase K digestion was too short.	Mix 200 µL whole blood thoroughly with 200 µL Lysis Buffer. After RNase treatment, add 20 µL Proteinase K and incubate at 55°C for the full 10 min; extend to 30 min when additional digestion is needed.
RNA contamination in purified DNA	RNase A was omitted, not reconstituted correctly, or the 3 min room-temperature incubation was not completed.	Add 20 µL reconstituted EcoSpin RNase A after Lysis Buffer addition, mix well, and incubate for 3 min at room temperature before adding Proteinase K.
Column flow is slow or sample remains on the membrane	The sample was overloaded, the standard blood input was exceeded, or lysis/digestion was incomplete.	Keep the direct whole-blood input at 200 µL. For larger blood volumes, use the RBC lysis workflow first. Confirm complete lysis and Proteinase K digestion before adding 400 µL Binding Buffer and loading the column. Centrifuge for 1 min at maximum speed.
Variable yield between blood preparations	Differences in blood input, reagent volumes, Proteinase K incubation, wash timing, centrifugation, or elution conditions.	Standardize each preparation to 200 µL whole blood and keep all protocol volumes and timing identical: 200 µL Lysis Buffer, 20 µL RNase A, 20 µL Proteinase K, 400 µL Binding Buffer, the specified two wash steps, and 30-50 µL elution.
Poor purity or inhibition in PCR/qPCR/Southern blotting/enzymatic reactions	Residual wash components remained on the membrane because Wash Buffer preparation or the final wash/dry-spin sequence was incomplete.	Confirm correct ethanol addition 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 centrifugation 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 the prepared buffer thoroughly and use 400 µL per column as specified in the protocol.
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 centrifugation.

Table continues on next page.

EcoSpin Blood 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 step, add 200 µL Wash Buffer 2 and centrifuge the column at maximum speed for the full 2 min. Transfer the column to a clean 1.5 mL tube before elution.
Low DNA concentration in the eluate	The elution volume was unnecessarily large, Elution Buffer was not applied to the membrane center, or the 5 min incubation was skipped.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the 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 contact the membrane adequately, the incubation was too short, or centrifugation was insufficient.	Transfer the column to a clean 1.5 mL tube, add 30-50 µL Elution Buffer to the membrane center, incubate 5 min at room temperature, and centrifuge 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 under 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 beyond 1 year, keep it at -20°C.
RNase A performance is reduced	Lyophilized RNase A was not reconstituted correctly or the prepared solution was stored outside the specified conditions.	Reconstitute RNase A in 1.1 mL RNase Reconstitution Buffer. The solution is stable for 1 year at 4°C; for storage beyond 1 year, keep it at -20°C.
Results remain inconsistent despite correct reagent volumes	One or more centrifugation or incubation steps differed from the protocol, especially the 1 min binding spin, Wash Buffer 1 spin, 30 sec Wash Buffer 2 spin, final 2 min wash spin, or 5 min elution incubation.	Repeat the workflow using the specified times and maximum-speed centrifugation conditions at room temperature. Keep incubation and spin timing identical across all samples being compared.

Still having trouble? Confirm both Wash Buffer ethanol additions, Proteinase K and RNase A reconstitution/storage, the standard 200 µL whole-blood input, 55°C digestion time, the complete 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 blood volume, any RBC lysis pretreatment, incubation times, centrifugation conditions, and observed DNA yield or downstream performance.