

EcoSpin Insect Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1080

Use this guide for common problems encountered during genomic DNA purification from insect samples. For best results, follow the specified 30-50 mg starting-material range, liquid-nitrogen grinding, 55°C Proteinase K digestion, clarification, 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 30-50 mg insect material per standard preparation. The datasheet notes a typical DNA yield of up to 20 µg from 50 mg insect material.
4. Grind the sample thoroughly in liquid nitrogen, then add 200 µL Resuspension Buffer followed by 200 µL Tissue Lysis Buffer and 20 µL Proteinase K.
5. Incubate for 3 h at 55°C, then centrifuge for 1 min at maximum speed and transfer the supernatant to a new tube.
6. Add 20 µL RNase A and incubate 3 min at room temperature, then add 400 µL Binding Buffer and 200 µL absolute ethanol and mix well.
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	Insect input was outside the standard 30-50 mg range; grinding was incomplete; Proteinase K digestion was insufficient; or the binding/wash/elution sequence was not completed as specified.	Use 30-50 mg insect material per standard preparation. Grind thoroughly in liquid nitrogen, add 200 µL Resuspension Buffer, then 200 µL Tissue Lysis Buffer plus 20 µL Proteinase K and incubate for 3 h at 55°C. Complete clarification, RNase A treatment, binding, washing, and 30-50 µL elution exactly as described.
Yield is lower than expected for a 50 mg sample	The insect was not ground to a sufficiently fine material, digestion was incomplete, or material was lost during clarification and supernatant transfer.	Grind the insect thoroughly in liquid nitrogen before adding buffer. Complete the full 3 h Proteinase K incubation at 55°C. After the 1 min maximum-speed clarification spin, transfer the supernatant carefully to a new tube without discarding it.
Poor results when processing more than 50 mg insect material	A starting amount above the stated 30-50 mg range was processed with the standard reagent volumes.	For more than 50 mg sample, scale up the amounts of reagents used in the entire protocol proportionally, as specified in the datasheet.
Insect tissue remains coarse or incompletely homogenized	Liquid-nitrogen grinding was incomplete before addition of Resuspension Buffer.	Grind the sample thoroughly in liquid nitrogen before transferring it to the microcentrifuge tube. Then add 200 µL EcoSpin Resuspension Buffer and mix before proceeding to Tissue Lysis Buffer and Proteinase K.
Incomplete lysis / visible tissue fragments remain	Proteinase K digestion at 55°C was shortened or the sample was not mixed thoroughly with Tissue Lysis Buffer.	Add 200 µL EcoSpin Tissue Lysis Buffer and 20 µL Proteinase K, mix well, and incubate for the full 3 h at 55°C before the clarification spin.
Column flow is slow or the membrane clogs	Particulate material remained because the sample was not fully ground/lysed or the clarified supernatant was not cleanly separated from debris.	After the 3 h digestion, centrifuge for 1 min at maximum speed at room temperature and transfer only the supernatant to a new 1.5 mL tube. Avoid transferring insoluble debris to the binding step.
Low yield after clarification	The supernatant containing the DNA-containing lysate was partially discarded or not fully transferred after centrifugation.	After the 1 min maximum-speed spin, retain and transfer the supernatant to a new tube as stated in the protocol. Proceed with RNase A treatment before adding Binding Buffer and ethanol.
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 to the clarified supernatant 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 followed by 200 µL absolute ethanol and mix well before loading the EcoSpin Column.
Sample remains on the column after the binding spin	The lysate contains residual debris or the centrifugation step was not performed at maximum speed for the full 1 min.	Use only the clarified supernatant for binding, load the sample after complete mixing with Binding Buffer and ethanol, and centrifuge for 1 min at maximum speed at room temperature.
Poor purity or inhibition in downstream PCR/qPCR/Southern blotting/enzymatic reactions	Wash Buffers were not prepared with ethanol 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 to remove residual wash buffer.

Table continues on next page.

EcoSpin Insect Genomic DNA Kit Troubleshooting Guide (continued)

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 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. 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 a larger volume than needed 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 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 for 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 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 insect samples	Differences in starting mass, grinding efficiency, lysis, supernatant recovery, reagent volumes, wash preparation, or elution conditions.	Standardize the workflow: use 30-50 mg input, complete liquid-nitrogen grinding, 3 h lysis at 55°C, clean supernatant transfer, the specified binding and wash volumes, the full 2 min final wash spin, and consistent 30-50 µL elution.

Still having trouble? Confirm the 30-50 mg insect input range, complete liquid-nitrogen grinding, the full 3 h Proteinase K incubation at 55°C, clean transfer of the clarified supernatant, RNase A treatment, correct Binding Buffer and ethanol volumes, both Wash Buffer ethanol additions, the final 2 min wash spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the insect mass, grinding/lysis details, reagent volumes, centrifugation conditions, and observed DNA yield or downstream performance.