

EcoPURE Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1075

Use this guide for common problems encountered during genomic DNA isolation from whole blood, cultured cells, fresh or frozen tissue and rodent tail, yeast, bacteria, insects, dried blood spots, swabs, and feather samples. The recommendations below follow the sample limits, lysis, digestion, binding, wash, centrifugation, and elution conditions specified in the product datasheet.

Before You Start - Quick Checks

1. Add 8.8 mL absolute ethanol to EcoPURE Wash Buffer 1 and 32 mL absolute ethanol to EcoPURE 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. The reconstituted solutions are stable for 1 year at 4°C; for storage longer than 1 year, store at -20°C.
3. Standard sample inputs include: 200 µL whole blood; 10⁴-10⁶ cells; up to 25 mg tissue; 0.6-1.2 cm mouse tail or 0.6 cm rat tail; up to 10⁸ yeast cells; 1 mL overnight bacterial culture; 30-50 mg insect; at least three 3 mm dried-blood-spot punches; swabs; and up to 50-80 mg feather.
4. Keep the sample-specific lysis conditions: Proteinase K digestion is typically at 55°C, while tissue/tail, insect, dried blood spot, swab, and feather workflows include additional sample-specific preparation or heating steps.
5. Use the protocol-specific Binding Buffer and ethanol sequence. Tissue/tail, insect, swab, and feather workflows require 200 µL absolute ethanol after 400 µL Binding Buffer; blood, cells, yeast, and bacterial workflows should be followed exactly as written in their respective protocols.
6. Follow the sample-specific wash sequence. Most protocols use Wash Buffer 1 followed by Wash Buffer 2; the bacterial workflow in this datasheet uses Wash Buffer 2 without a Wash Buffer 1 step.
7. Complete the final 200 µL Wash Buffer 2 step with a 2 minute maximum-speed spin to remove residual wash buffer.
8. Elute with 30-50 µL EcoPURE Elution Buffer applied to the membrane center; incubate 5 minutes at room temperature, then centrifuge 30 seconds at maximum speed.

Application	Problem	Most likely cause	Recommended action
General	Low or inconsistent DNA yield	Incomplete sample lysis, incorrect sample-to-reagent scaling, shortened Proteinase K incubation, incomplete column loading, or inconsistent elution conditions.	Use the sample-specific input limit and reagent volumes in the datasheet. Ensure complete lysis before binding/loading, use the specified Proteinase K incubation, transfer the complete prepared sample to the column, and elute with 30-50 µL Elution Buffer after the 5 minute room-temperature incubation.
General	Wash performance is poor / downstream inhibition	Wash Buffer 1 or Wash Buffer 2 was used before ethanol was added, or the final Wash Buffer 2 high-speed spin was shortened.	Before first use add 8.8 mL absolute ethanol to Wash Buffer 1 and 32 mL absolute ethanol to Wash Buffer 2. Follow the sample-specific wash sequence exactly and complete the final 200 µL Wash Buffer 2 step with the stated 2 minute maximum-speed centrifugation.
General	RNA contamination in the DNA preparation	RNase A treatment was omitted or the reconstituted RNase A was not handled/stored as specified.	Use the 20 µL RNase A treatment where specified in the sample protocol and incubate for 3 minutes at room temperature. Reconstitute lyophilized RNase A in 1.1 mL RNase Reconstitution Buffer and store the solution according to the datasheet.
General	Protein digestion appears incomplete	Proteinase K was not reconstituted correctly, incubation at 55°C was shortened, or a difficult sample required longer digestion.	Reconstitute lyophilized Proteinase K in 1.1 mL Proteinase K Storage Buffer. Use 20 µL per standard preparation where specified. For blood, cells, yeast, bacteria and dried blood spots, the datasheet notes that extending the 55°C incubation from 10 to 30 minutes can increase yield.
General	Low DNA concentration in the eluate	Elution Buffer was not applied to the membrane center, the 5 minute incubation was omitted, or a larger elution volume than the stated range was used.	Add 30-50 µL EcoPURE Elution Buffer to the center of the membrane, incubate for 5 minutes at room temperature, then centrifuge 30 seconds at maximum speed.
General	Column flow is slow or sample remains on the membrane	The sample was not completely lysed or homogenized, solid material remained, or more input material was processed without proportional reagent scaling.	Complete the sample-specific lysis/homogenization step before loading. Stay within the stated sample input or scale the entire protocol proportionally when the datasheet permits it. For workflows that generate a clarified supernatant, transfer only the specified supernatant to the next step.
Blood	Low DNA recovery from whole blood	More than 200 µL blood was processed using the standard procedure, or the lysis/Proteinase K incubation was incomplete.	Use 200 µL whole blood for the standard protocol, add 200 µL Lysis Buffer, 20 µL RNase A, and 20 µL Proteinase K, then incubate 10 minutes at 55°C before adding 400 µL Binding Buffer. The Proteinase K incubation may be extended to 30 minutes to increase yield.
Blood	Processing a blood volume greater than 200 µL	The standard Genomic DNA Kit blood workflow is designed for 200 µL whole blood.	For higher whole-blood volumes, the datasheet directs use of EcoTech Red Blood Cell Lysis Buffer (10x, Cat No: RBCLB-10x) to isolate white blood cells from up to 9 mL blood, then continue the genomic DNA workflow with those cells.

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Application	Problem	Most likely cause	Recommended action
Cells	Cell pellet is difficult to lyse / visible clumps remain	The pellet was not fully resuspended before Lysis Buffer addition.	For 10 ⁴ -10 ⁶ cells, resuspend the pellet completely in 200 µL Resuspension Buffer by vortexing or pipetting until no clumps remain, then add 200 µL Lysis Buffer and continue with RNase A and Proteinase K treatment.
Cells	Low yield when using more than 10 ⁶ cells	Cell input exceeded the standard range without proportional reagent scaling.	The standard cell protocol is for 10 ⁴ to 10 ⁶ cells. If more cells are required, scale the amounts of reagents used in the entire protocol proportionally as stated in the datasheet.
Tissue / tail	Tissue or rodent tail is not completely lysed	The sample pieces were too large, tissue was not pre-ground when possible, or the 55°C Proteinase K digestion was stopped too early.	Cut tissue/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 completely lysed. Typical lysis is 1-3 h for tissue and 6-8 h for rodent tails.
Tissue / tail	Tissue lysis is taking too long	Solid sample size/structure slows enzymatic digestion.	Vortex the sample every 15 minutes during the 55°C incubation as suggested in the datasheet. Cutting into smaller pieces or grinding in liquid nitrogen can also reduce lysis time.
Tissue / tail	Low DNA recovery from tissue or tail	Sample input exceeded the standard limit, lysis was incomplete, or the Binding Buffer/ethanol step was incomplete.	Use up to 25 mg fresh/frozen tissue, 0.6-1.2 cm mouse tail, or 0.6 cm rat tail for the standard protocol. After complete lysis and RNase A treatment, add 400 µL Binding Buffer followed by 200 µL absolute ethanol and mix well before column loading.
Yeast	Low DNA recovery from yeast	Yeast cell-wall digestion was omitted/incomplete, the pellet remained clumped, or more than 10 ⁸ cells were processed without scaling.	Resuspend the yeast pellet in 200 µL Resuspension Buffer until no clumps remain. Add 10 µL lyticase (0.5 mg/mL, not provided) and incubate 30 minutes at 37°C before continuing. For more than 10 ⁸ cells, scale the entire protocol proportionally.
Yeast	Yeast lysate remains difficult to process	The lyticase step was shortened or cell clumps remained before lysis.	Repeat thorough resuspension by vortexing or pipetting until no clumps remain and complete the full 30 minute lyticase incubation at 37°C before adding Lysis Buffer.
Bacteria	Low DNA recovery from gram-positive bacteria	The thick peptidoglycan cell wall was not sufficiently disrupted.	For most gram-positive bacteria, use the kit together with lysozyme enzyme (not provided), as directed in the datasheet, before continuing with the standard lysis/binding workflow.
Bacteria	Bacterial pellet does not resuspend completely	The pellet from the 1 mL overnight culture remained clumped after centrifugation.	After harvesting 1 mL overnight culture at 6000 rpm for 3 minutes, resuspend the pellet in 200 µL Resuspension Buffer by vortexing or pipetting until no cell clumps remain before adding Lysis Buffer.
Bacteria	Variable bacterial DNA yield between cultures	Different culture volumes were processed with unchanged reagent volumes, or Proteinase K incubation differed between samples.	The standard procedure is for 1 mL overnight bacterial culture. For higher volumes, scale the reagents proportionally. Keep the 20 µL Proteinase K / 55°C incubation consistent; extending to 30 minutes may increase yield.
Bacteria	Wash sequence differs from other sample protocols	The bacterial workflow was assumed to use Wash Buffer 1 because most other sample protocols do.	Follow the bacterial protocol as written: after column binding, add 500 µL Wash Buffer 2, centrifuge 30 seconds, then add 200 µL Wash Buffer 2 and centrifuge 2 minutes at maximum speed. The supplied bacterial protocol does not include a Wash Buffer 1 step.
Insect	Low DNA yield from insect tissue	Sample was not finely ground, the 55°C digestion was incomplete, or the clarified supernatant was not transferred cleanly.	Use 30-50 mg insect, grind in liquid nitrogen, add Resuspension Buffer/Tissue Lysis Buffer and 20 µL Proteinase K, and incubate at 55°C for 3 hours. Vortex every 15 minutes if needed. Centrifuge 1 minute at maximum speed and transfer the supernatant to a new tube before RNase A treatment.
Dried blood spot	Low DNA recovery from dried blood spots	Too little spot material was used or one of the sequential heating/digestion steps was shortened.	Use at least three 3 mm punched-out circles. Incubate with Tissue Lysis Buffer at 85°C for 10 minutes, then add 20 µL Proteinase K and incubate 10 minutes at 55°C. After adding 400 µL Binding Buffer, incubate at 70°C for 10 minutes before RNase A treatment and column loading.
Swab	Low DNA recovery from swabs	The cotton tip was not processed fully, the initial Tissue Lysis Buffer incubation was shortened, or the supernatant was not transferred before Proteinase K digestion.	Using sterile technique, cut the cotton tip into 200 µL Resuspension Buffer. Add 200 µL Tissue Lysis Buffer, vortex gently, incubate 5 minutes at room temperature, transfer the supernatant to a clean tube, then add 20 µL Proteinase K and incubate at 55°C until cells are completely lysed.
Feather	Low DNA yield from feather samples	Sample exceeded 50-80 mg, feather pieces were too large, reducing agent was omitted, or lysis remained incomplete.	Use up to 50-80 mg feather and cut into 0.1-1 mm pieces or grind in liquid nitrogen. Add 20 µL 1 M DTT per 50 mg sample (or 40 µL beta-mercaptoethanol instead), then add Tissue Lysis Buffer and Proteinase K and incubate at 55°C/90 rpm until homogeneous.

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Feather	Large quill particles remain after lysis	Solid quill material requires longer digestion than softer feather material.	For larger solid quill particles, leave the sample in a shaker at 55°C and 90 rpm overnight, as specified in the datasheet.
Feather	Two phases remain after Binding Buffer addition	The feather lysate can transiently separate into two phases at this step.	After adding 400 µL Binding Buffer, mix thoroughly until a homogeneous appearance is achieved. Then incubate at 70°C/90 rpm for 10 minutes, vortexing 60 seconds every 3 minutes, before adding 200 µL absolute 99-100% ethanol.
Feather	Prepared feather lysate volume exceeds one column load	The complete mixture cannot be transferred to the EcoPURE Column in one loading step.	Load the prepared mixture onto the same column, centrifuge 1 minute at maximum speed at room temperature, and repeat loading with the same column until the entire mixture has been processed.
Feather	Dark-colored mixture or DNA is observed	Pigmented material from dark hair/feather-type samples can produce a dark appearance.	The datasheet notes that dark color in the mixture and DNA can be a normal observation for dark hair-type material. Continue the protocol if the other processing steps are proceeding normally.

Still having trouble? Re-check the sample input, complete sample-specific lysis/digestion, Wash Buffer ethanol preparation, Binding Buffer and ethanol sequence, wash order, final 2 minute 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, reagent volumes, incubation conditions, centrifugation conditions, and observed DNA yield or downstream issue.