

EcoPURE Total RNA Kit

Troubleshooting Guide

50 rxns

Cat No: E2075

Use this guide for common problems encountered during total RNA isolation from whole blood, suspension or adherent cultured cells, and fresh or frozen tissue. The recommendations below follow the sample limits, lysis, ethanol, wash, centrifugation, and elution conditions specified in the product datasheet.

Before You Start - Quick Checks

1. Add 40 mL absolute ethanol to the 10 mL EcoPURE Wash Buffer 2 concentrate before first use.
2. Standard inputs are: up to 100 µL fresh EDTA whole blood; 1x10⁴ to 2x10⁶ cultured cells; or up to 30 mg fresh/frozen tissue.
3. Use 400 µL EcoPURE Lysis/Binding Buffer for the standard whole-blood, cell, and tissue workflows.
4. For whole blood and cells, add 400 µL absolute ethanol after lysis. For tissue, first clarify the lysate, note the supernatant volume, then add an equal volume of absolute ethanol.
5. Load the column in 700 µL portions as needed and centrifuge 30 seconds at maximum speed at room temperature.
6. For maximum removal of residual genomic DNA in sensitive downstream assays, use the optional On-Column DNA Removal step.
7. Wash sequentially with 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, then 200 µL Wash Buffer 2 followed by a 2 minute maximum-speed spin.
8. Elute from the membrane center after 1 minute incubation: 30-50 µL for whole blood, and 50-100 µL for cultured cells or tissue.
9. For samples with high RNase activity, the datasheet allows 4 µL beta-mercaptoethanol per 400 µL Lysis/Binding Buffer. For >1x10⁶ cells or viscous tissue lysates, optional 25 gauge needle shearing (5-10 passes) may be used.

Application	Problem	Most likely cause	Recommended action
General	Low or no RNA yield	Incomplete lysis or homogenization; incorrect ethanol addition; sample input above the stated capacity without proportional reagent scaling; incomplete transfer to the column; or inconsistent elution.	Use the protocol-specific input limits and reagent ratios. Ensure complete lysis before ethanol addition, transfer the entire lysate in 700 µL loading portions as needed, complete all wash steps, and elute from the center of the membrane after the 1 minute incubation.
General	Variable yield between samples	Differences in sample amount, lysis efficiency, ethanol volume, centrifugation, wash steps, or elution volume.	Standardize sample input and keep all protocol steps consistent. Scale reagents proportionally only when the datasheet specifically allows it, and use the same centrifugation and elution conditions for samples being compared.
General	RNA degradation / poor integrity	RNase exposure, delayed lysis, thawing of frozen cell pellets before lysis, tissue thawing during preparation, or prolonged storage of frozen cell pellets.	Use RNase-free tubes and handling. Lyse samples promptly. Do not thaw frozen cell pellets before adding Lysis/Binding Buffer, and keep liquid-nitrogen-ground tissue from thawing before lysis. Frozen cell pellets should not be stored longer than the 2 week period stated in the protocol.
General	Genomic DNA contamination	Residual genomic DNA remains in the RNA preparation, particularly when downstream assays are highly sensitive.	Apply the optional On-Column DNA Removal procedure when maximum removal of residual DNA is required for sensitive downstream applications.
General	Residual wash buffer / downstream inhibition	Wash Buffer 2 was not prepared with ethanol, or the final 200 µL Wash Buffer 2 step and 2 minute high-speed spin were incomplete.	Before first use, add 40 mL absolute ethanol to the 10 mL Wash Buffer 2 concentrate. Follow the 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, and final 200 µL Wash Buffer 2 steps, including the 2 minute maximum-speed spin.
General	Low RNA concentration in eluate	Elution volume is larger than needed for the sample type, or Elution Buffer was not placed on the center of the membrane.	Use the datasheet elution range for the sample type: 30-50 µL for whole blood and 50-100 µL for cultured cells or tissue. Apply buffer to the center of the membrane and incubate 1 minute before centrifugation.
General	Column flow is slow / column appears overloaded	Too much sample was loaded at once, lysate remains viscous, tissue debris was carried forward, or sample input exceeded the stated protocol capacity.	Load up to 700 µL at a time and repeat loading as necessary. For high-cell-number or tissue lysates, use the protocol-recommended DNA shearing step when needed. Clarify tissue lysate by the specified 2 minute maximum-speed spin before ethanol addition and column loading.
General	Viscous lysate	High genomic DNA content, especially with more than 1x10 ⁶ cells or tissue lysates.	For more than 1x10 ⁶ cells, pass the lysate through a 25 gauge needle 5-10 times before loading. The tissue protocol also recommends this optional step to shear genomic DNA and reduce viscosity.
Whole blood	Incomplete blood lysis / mixture does not become brownish black	Lysis/Binding Buffer was insufficient, mixing was incomplete, or the 10 second vortex step was shortened.	For each 100 µL fresh EDTA whole-blood sample, add 400 µL Lysis/Binding Buffer, mix by pipetting, then vortex for 10 seconds until the mixture becomes brownish black before adding ethanol.

Table continues on next page.

EcoPURE Total RNA Kit Troubleshooting Guide (continued)

Application	Problem	Most likely cause	Recommended action
Whole blood	Poor recovery from whole blood	More than 100 µL whole blood was processed with the standard protocol, the sample was not fresh/non-coagulating, or ethanol was not added at the required volume.	Use up to 100 µL fresh non-coagulating whole blood collected with EDTA. Add 400 µL Lysis/Binding Buffer followed by 400 µL absolute ethanol. For higher blood volumes, use the EcoSpin Blood Total RNA Kit (E2090) as directed in the datasheet.
Whole blood	Inconsistent binding after ethanol addition	The 400 µL absolute ethanol addition was omitted or mixing after ethanol addition was incomplete.	Add 400 µL absolute 96-100% ethanol to the lysate and vortex for 10 seconds before transferring the sample to the EcoPURE Column.
Cells	Cell pellet does not fully lyse	Cell pellet was not completely dispersed in Lysis/Binding Buffer before the ethanol step.	Add 400 µL Lysis/Binding Buffer to the pelleted cells, mix well by pipetting, and vortex for 10 seconds until the entire pellet is completely dissolved before proceeding.
Cells	Low yield from large cell numbers	More than 2×10^6 cells were processed without scaling the protocol, or the lysate was too viscous for efficient column loading.	The standard procedure is for 1×10^4 to 2×10^6 cells. If more cells are required, scale the reagents in the entire protocol proportionally. For more than 1×10^6 cells, consider the optional 25 gauge needle shearing step.
Cells	RNA quality decreases with frozen cell pellets	Frozen pellets were stored too long or thawed before lysis.	Store cell pellets at -80°C for no longer than 2 weeks as stated in the datasheet. Do not thaw frozen pellets before extraction; immediately add Lysis/Binding Buffer and begin lysis.
Cells / Tissue	High-RNase samples give poor RNA quality	Endogenous RNase activity is unusually high for the sample type.	The datasheet states that 4 µL beta-mercaptoethanol may be added to each 400 µL Lysis/Binding Buffer for samples known to have high RNase activity to help ensure RNase inactivation.
Tissue	Low yield from tissue	Incomplete grinding or homogenization, tissue input above 30 mg without scaling, or incomplete recovery of clarified lysate.	Use up to 30 mg fresh or frozen tissue for the standard procedure. Homogenize thoroughly by the liquid-nitrogen or rotor-stator method, clarify the lysate for 2 minutes at maximum speed, transfer the supernatant, and continue using the measured supernatant volume.
Tissue	Tissue thaws during preparation	Liquid nitrogen evaporated and the ground tissue was allowed to warm before Lysis/Binding Buffer was added.	When using the liquid-nitrogen method, allow the liquid nitrogen to evaporate without allowing the tissue to thaw, then immediately add 400 µL Lysis/Binding Buffer and transfer to an RNase-free tube.
Tissue	Incomplete tissue homogenization	Grinding was insufficient or rotor-stator homogenization was too brief or performed at the wrong speed.	For the rotor-stator route, add 400 µL Lysis/Binding Buffer and homogenize immediately and vigorously at 15,000 rpm for 30 seconds. For the liquid-nitrogen route, grind the sample thoroughly before lysis.
Tissue	Debris carried onto the column	The clarification spin after tissue homogenization was omitted or the pellet was disturbed while transferring supernatant.	Centrifuge the tissue lysate for 2 minutes at maximum speed at room temperature to pellet debris. Transfer only the supernatant to a new RNase-free tube and note its volume before adding ethanol.
Tissue	Poor binding after tissue clarification	Ethanol was not added in an amount equal to the measured supernatant volume.	After clarification, note the supernatant volume and add an equal volume of absolute 96-100% ethanol. Mix well by vortexing for 10 seconds before loading the column.
Tissue	Low yield with more than 30 mg tissue	Sample amount exceeded the standard tissue input and reagent volumes were not scaled proportionally.	The standard procedure is for up to 30 mg tissue. If more tissue is required, scale the amounts of reagents used in the entire protocol proportionally, as stated in the datasheet.

Still having trouble? Re-check the sample input, complete lysis or homogenization, ethanol volume, Wash Buffer 2 ethanol preparation, loading volume, the final 2 minute Wash Buffer 2 spin, and the sample-specific elution range. If the problem persists, contact EcoTech Biotechnology technical support with the sample type and amount, reagent volumes used, centrifugation conditions, elution volume, and observed RNA yield or downstream issue.