

EcoPURE Total RNA Micro Kit

Troubleshooting Guide

50 rxns

Cat No: E2416

Use this guide for common problems encountered during micro-scale total RNA isolation from 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 Micro Wash Buffer 2 concentrate before first use.
2. Standard input ranges are 1×10^3 to 2.5×10^5 cultured cells or up to 3-5 mg fresh/frozen tissue.
3. For low cell inputs ($< 1 \times 10^5$ cells), 30-50 μ L medium may be left behind to avoid losing an invisible pellet.
4. Add 400 μ L EcoPURE Micro Lysis/Binding Buffer and ensure complete lysis before ethanol addition.
5. For cultured cells, add 400 μ L absolute ethanol. For tissue, clarify the lysate first, note the supernatant volume, then add an equal volume of absolute ethanol.
6. Load the Micro Spin Column in 700 μ L portions as needed and centrifuge 30 seconds at maximum speed at room temperature.
7. For maximum removal of residual genomic DNA in sensitive downstream assays, use the optional On-Column DNA Removal step.
8. Wash sequentially with 400 μ L Micro Wash Buffer 1, 500 μ L Micro Wash Buffer 2, then 200 μ L Micro Wash Buffer 2 followed by a 2 minute maximum-speed spin.
9. Elute with 10-20 μ L EcoPURE Micro Elution Buffer applied to the membrane center; incubate 1 minute before centrifugation.

Application	Problem	Most likely cause	Recommended action
General	Low or no RNA yield	Incomplete lysis or homogenization; ethanol addition incorrect; incomplete transfer to the Micro Spin Column; or elution conditions were not followed.	Use the sample-specific input limits and reagent volumes. Ensure complete lysis before ethanol addition, transfer the lysate in 700 μ L 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 input, pellet recovery, lysis efficiency, ethanol volume, wash sequence, centrifugation, or elution volume.	Standardize the starting material and keep lysis, ethanol addition, loading, wash, centrifugation, and 10-20 μ L elution conditions consistent between samples.
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 Micro Lysis/Binding Buffer, and do not allow liquid-nitrogen-ground tissue to thaw before lysis.
General	Genomic DNA contamination	Residual genomic DNA remains in the RNA preparation and affects sensitive downstream assays.	Use the optional On-Column DNA Removal procedure when maximum removal of residual DNA is required.
General	Poor downstream performance / residual wash components	Micro Wash Buffer 2 was not prepared with ethanol, or the final 200 μ L Micro Wash Buffer 2 step and 2 minute maximum-speed spin were incomplete.	Before first use, add 40 mL absolute ethanol to the 10 mL Micro Wash Buffer 2 concentrate. Follow the 400 μ L Micro Wash Buffer 1, 500 μ L Micro Wash Buffer 2, and final 200 μ L Micro Wash Buffer 2 steps, including the 2 minute maximum-speed spin.
General	Low RNA concentration in eluate	Elution volume was larger than the specified micro-scale range, or Elution Buffer was not applied to the center of the membrane.	Use 10-20 μ L EcoPURE Micro Elution Buffer, apply it directly to the center of the membrane, incubate 1 minute at room temperature, then centrifuge 30 seconds at maximum speed.
General	Column flow is slow / liquid remains on the membrane	Lysate contains debris, the sample is too viscous, too much material was loaded, or more than 700 μ L was loaded at one time.	Use the stated sample limits, ensure complete lysis or homogenization, clarify tissue lysate as specified, and load the Micro Spin Column in 700 μ L portions, repeating the loading step if necessary.
Cells	Very small or invisible cell pellet is lost	Supernatant was removed too aggressively from a low-cell-number sample.	For inputs below 1×10^5 cells, the datasheet allows 30-50 μ L of media to remain so that an invisible pellet is not dislodged. Decant or aspirate carefully before lysis.
Cells	Cell pellet does not fully lyse	The pellet was not completely dispersed in Micro Lysis/Binding Buffer before the ethanol step.	Add 400 μ L EcoPURE Micro Lysis/Binding Buffer, mix well by pipetting, and vortex for 10 seconds until the entire pellet is completely dissolved before proceeding.
Cells	Low yield from very low cell input	The small pellet was lost during washing or supernatant removal, or sample handling was inconsistent.	Handle low-input pellets carefully. For fewer than 1×10^5 cells, leave 30-50 μ L medium if needed to protect the pellet, then add the full 400 μ L Micro Lysis/Binding Buffer and continue with the standard protocol.
Cells	Poor recovery when cell input is high	The sample exceeded the stated range of 1×10^3 to 2.5×10^5 cells, increasing lysate load and column burden.	Use the Micro Kit within the stated 1×10^3 to 2.5×10^5 cell range. Avoid loading more cells than specified in the datasheet.

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Troubleshooting Guide (continued)

Application	Problem	Most likely cause	Recommended action
Cells	Frozen cell pellet gives degraded RNA	The pellet was stored longer than specified or thawed before lysis.	Store frozen cell pellets at -80°C for no longer than 2 weeks as specified. Do not thaw before extraction; immediately add EcoPURE Micro Lysis/Binding Buffer to the frozen pellet.
Cells / Tissue	High-RNase sample gives poor RNA integrity	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 EcoPURE Micro Lysis/Binding Buffer for samples known to have high RNase activity.
Cells	Poor binding after ethanol addition	The 400 µL absolute ethanol addition was omitted or mixing after ethanol addition was incomplete.	After complete cell lysis, add 400 µL absolute 96-100% ethanol and vortex for 10 seconds before loading the Micro Spin Column.
Tissue	Low RNA yield from tissue	Tissue input exceeded 3-5 mg, grinding or homogenization was incomplete, debris was carried forward, or ethanol was not added in the correct proportion.	Use up to 3-5 mg fresh or frozen tissue. Homogenize thoroughly, clarify the lysate for 2 minutes at maximum speed, transfer the supernatant, note its volume, and add an equal volume of absolute ethanol before column loading.
Tissue	Tissue thaws during liquid-nitrogen preparation	The ground tissue warmed before EcoPURE Micro Lysis/Binding Buffer was added.	Allow liquid nitrogen to evaporate without allowing the tissue to thaw, then immediately add 400 µL EcoPURE Micro Lysis/Binding Buffer and transfer the homogenized sample to an RNase-free tube.
Tissue	Incomplete tissue homogenization	Grinding was insufficient or rotor-stator homogenization was not performed at the specified conditions.	For the rotor-stator route, add 400 µL Micro Lysis/Binding Buffer and homogenize immediately and vigorously at 15,000 rpm for 30 seconds. For the liquid-nitrogen route, grind thoroughly before lysis.
Tissue	Debris carried onto the Micro Spin Column	The 2 minute clarification spin after tissue homogenization was omitted, or the debris pellet was disturbed during transfer.	Centrifuge the lysate for 2 minutes at maximum speed at room temperature, transfer only the supernatant to a new RNase-free tube, and note the supernatant volume before ethanol addition.
Tissue	Poor binding after tissue clarification	Absolute 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. Vortex for 10 seconds before loading the column.
General	Inconsistent elution between samples	Elution Buffer placement, incubation time, or volume differed between samples.	Use 10-20 µL EcoPURE Micro Elution Buffer for each sample, apply it to the membrane center, incubate 1 minute at room temperature, and centrifuge 30 seconds at maximum speed.

Still having trouble? Re-check the sample input, complete lysis or homogenization, ethanol volume, Micro Wash Buffer 2 ethanol preparation, 700 µL loading portions, the final 2 minute wash spin, and the 10-20 µL elution step. 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.