

EcoSpin Liquid Sample Total RNA Kit

Troubleshooting Guide

50 rxns

Cat No: E4075

Use this guide for common problems encountered during total RNA purification from liquid samples such as saliva, serum, plasma, and urine. For best results, follow the specified sample-to-lysis-buffer ratio, chloroform phase-separation conditions, careful upper-phase transfer, isopropanol addition, column loading, wash, dry-spin, and elution steps in the product protocol.

Before You Start - Quick Checks

1. Store EcoSpin LS Lysis/Binding Buffer at 4-8°C upon receipt; store the remaining kit components at 15-25°C.
2. Use 250 µL liquid sample per preparation. Add 750 µL EcoSpin LS Lysis/Binding Buffer and pipette up and down several times until completely homogeneous.
3. After homogenization, samples may be stored at 4°C overnight or at -70°C for up to one year. Otherwise, continue directly with the 5 min room-temperature lysis incubation.
4. Add 200 µL chloroform, mix by shaking, incubate 3 min at room temperature, then centrifuge at 12,000 rpm for 15 min at 4°C.
5. Transfer only the upper phase to a new RNase-free tube. Do not contaminate the upper phase with the lower phase or interface.
6. Add 500 µL isopropanol to the recovered upper phase. Load 700 µL per column spin and repeat the loading step as necessary.
7. Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before first use. Complete the 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, and final 200 µL Wash Buffer 2 / 2 min spin steps.
8. Elute with 30-50 µL EcoSpin Elution Buffer applied to the center of the membrane; incubate 1 min at room temperature before centrifugation.

Problem	Most likely cause	Recommended action
Low or no RNA yield	Sample volume exceeded 250 µL; the 1:3 sample-to-LS Lysis/Binding Buffer ratio was not followed; phase separation was incomplete; isopropanol was omitted; or the upper phase was not fully transferred to the column.	Use 250 µL liquid sample and add 750 µL EcoSpin LS Lysis/Binding Buffer. Homogenize completely, incubate 5 min at room temperature, perform the chloroform separation exactly as specified, transfer the upper phase carefully, add 500 µL isopropanol, and process the complete sample through the column in 700 µL portions.
Lysate is not homogeneous after addition of LS Lysis/Binding Buffer	The sample and 750 µL LS Lysis/Binding Buffer were not mixed sufficiently.	Pipette the lysate up and down several times until complete homogenization is achieved before the 5 min room-temperature incubation.
Phase separation is poor or upper phase is difficult to identify	Chloroform mixing, 3 min incubation, or the 12,000 rpm / 15 min / 4°C centrifugation conditions were not followed.	Add 200 µL chloroform, mix well by shaking, incubate 3 min at room temperature, and centrifuge at 12,000 rpm for 15 min at 4°C before collecting the upper phase.
Upper phase is contaminated with lower phase	The interphase/lower phase was disturbed during transfer after chloroform separation.	Transfer only the clear upper phase to a new RNase-free tube. Avoid aspirating material from the lower phase or interface, as specified in the protocol.
Low RNA recovery after phase transfer	Part of the upper phase was left behind, or 500 µL isopropanol was not added after transfer.	Carefully recover as much of the upper phase as possible without contaminating it with the lower phase, then add 500 µL isopropanol and mix well by shaking before column loading.
Column flow is slow or liquid remains on the membrane	More than 700 µL was loaded at once, or the phase transferred to the column contained material from the lower phase.	Load a maximum of 700 µL per column spin, centrifuge 30 sec at maximum speed at room temperature, and repeat as necessary. During phase transfer, avoid contamination from the lower phase.
RNA purity is poor / downstream inhibition	Lower-phase carryover, incomplete washing, Wash Buffer 2 not prepared with ethanol, or insufficient final 2 min spin left residual wash components on the membrane.	Collect only the upper phase. Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before first use. Perform the 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, and final 200 µL Wash Buffer 2 / 2 min maximum-speed steps exactly as specified.
Wash Buffer 2 gives inconsistent results	Absolute ethanol was not added to the Wash Buffer 2 concentrate before use.	Add 40 mL absolute ethanol to the 10 mL EcoSpin Wash Buffer 2 concentrate before first use and mix thoroughly.
Ethanol / wash-buffer carryover in the eluate	The final 200 µL Wash Buffer 2 step or the 2 min maximum-speed centrifugation was omitted or shortened.	After the 500 µL Wash Buffer 2 wash, add 200 µL Wash Buffer 2 and centrifuge the column for the full 2 min at maximum speed. Transfer the column to a clean RNase-free tube before elution.
Low RNA concentration in the eluate	Elution Buffer was not applied to the center of the membrane, the 1 min incubation was skipped, or the selected elution volume was larger than needed.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the membrane, incubate 1 min at room temperature, and centrifuge 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not contact the membrane adequately, incubation was insufficient, or the final centrifugation was incomplete.	Transfer the column to a clean RNase-free 1.5 mL tube, add 30-50 µL Elution Buffer to the membrane center, incubate for 1 min, and centrifuge 30 sec at maximum speed.

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Problem	Most likely cause	Recommended action
RNA integrity is poor	Sample handling was prolonged before lysis, RNase-free technique was not maintained, or the homogenized lysate was stored outside the conditions described in the protocol.	Use RNase-free tubes and process samples promptly. After homogenization, the protocol permits storage at 4°C overnight or at -70°C for up to one year; otherwise proceed directly with the 5 min room-temperature lysis step.
Variable results between saliva, serum, plasma, urine or other liquid samples	Different sample matrices were processed with inconsistent starting volume, mixing, phase separation, or transfer conditions.	Standardize the workflow at 250 µL sample input, 750 µL LS Lysis/Binding Buffer, 200 µL chloroform, 12,000 rpm for 15 min at 4°C, careful upper-phase transfer, and 500 µL isopropanol.
Unexpectedly low recovery after overnight / frozen lysate storage	The lysate was stored outside the conditions stated in the protocol or was not fully re-homogenized before continuing.	If pausing after homogenization, use the protocol-specified storage conditions: 4°C overnight or -70°C for up to one year. Before proceeding, ensure the lysate is fully homogeneous and continue with the 5 min room-temperature incubation.
Results remain inconsistent despite correct reagent volumes	One or more timing, temperature, centrifugation, phase-transfer, wash, or elution steps differed between runs.	Repeat the workflow using the specified 250 µL sample input, 750 µL LS Lysis/Binding Buffer, 5 min lysis, 200 µL chloroform, 3 min incubation, 12,000 rpm / 15 min / 4°C phase separation, 500 µL isopropanol, 700 µL loading portions, complete wash sequence, and 30-50 µL elution.

Still having trouble? Re-check the 250 µL sample input, 750 µL LS Lysis/Binding Buffer, complete homogenization, 5 min lysis, 200 µL chloroform addition, 3 min room-temperature incubation, 12,000 rpm / 15 min / 4°C phase separation, careful upper-phase transfer, 500 µL isopropanol addition, 700 µL column loading portions, Wash Buffer 2 ethanol preparation, final 2 min wash spin, and 30-50 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the sample type, starting volume, phase appearance, transfer details, centrifugation conditions, and observed RNA yield or downstream issue.