

EcoSpin Blood Total RNA Kit

Troubleshooting Guide

50 rxns

Cat No: E2090

Use this guide for common problems encountered during red blood cell lysis, mononuclear-cell recovery, and total RNA purification from whole blood. For best results, follow the specified RBCL dilution, blood-to-buffer ratio, gentle pellet handling, lysate homogenization, ethanol addition, wash, dry-spin, and elution conditions in the product protocol.

Before You Start - Quick Checks

1. Store EcoSpin RBCL Buffer at 4-8°C upon receipt. Prepare the 1x working solution before use by diluting 1 part RBCL Buffer with 9 parts room-temperature RNase-free water, then warm it to room temperature.
2. Use fresh whole blood at room temperature collected with EDTA or another anticoagulant; the protocol supports up to 7 mL whole blood per preparation.
3. Use approximately 1 part blood to 6 parts 1x RBCL Buffer. Mix by gentle inversion only, incubate 10 min at room temperature protected from light, and do not vortex.
4. Follow the 1500 rpm centrifugation steps carefully and remove supernatants without disturbing the recovered cell pellet. Complete the second RBCL treatment and final PBS wash before RNA isolation.
5. Add 400 µL EcoSpin Lysis/Binding Buffer and homogenize with a 20-gauge (0.9 mm) needle 5-10 times or until the lysate is homogeneous.
6. Add 400 µL absolute (96-100%) ethanol to the lysate. 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 500 µL and 200 µL Wash Buffer 2 steps, including the final 2 min maximum-speed spin.
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
Incomplete red blood cell lysis / red coloration remains	EcoSpin RBCL Buffer was diluted incorrectly; the 1x working solution or blood was not at room temperature; the approximately 1:6 blood-to-buffer ratio was not used; mixing or incubation was insufficient.	Prepare 1x RBCL Buffer using 1 part concentrate plus 9 parts room-temperature RNase-free water. Warm the working solution to room temperature, use fresh room-temperature blood, add approximately 6 parts 1x RBCL Buffer per 1 part blood, invert gently, and incubate for the full 10 min protected from light.
Cell pellet remains visibly red after the first RBCL centrifugation	Residual erythrocytes remain after the initial lysis step.	Carefully remove the clear red supernatant without disturbing the pellet. Gently resuspend the pellet in 1 mL diluted 1x EcoSpin RBCL Buffer, incubate 5 min at room temperature protected from light, then centrifuge at 1500 rpm for 3 min.
Low leukocyte / mononuclear-cell recovery	The pellet was disturbed or aspirated during supernatant removal, or handling was too vigorous during resuspension.	Remove supernatants carefully without disturbing the pellet. Resuspend gently and do not vortex during the RBCL steps. Follow the specified 1500 rpm centrifugation conditions and pellet-handling instructions.
Variable recovery between blood samples	Blood freshness, temperature, anticoagulant collection, RBCL working-solution preparation, blood-to-buffer ratio, incubation, mixing, or pellet handling differed between samples.	Standardize the workflow: use fresh anticoagulated blood at room temperature, freshly prepared 1x RBCL Buffer, an approximately 1:6 blood-to-buffer ratio, fixed incubation times, gentle inversion, and consistent centrifugation and pellet handling.
Low total RNA yield	Mononuclear-cell recovery was low; the recovered pellet was not completely lysed; homogenization was insufficient; ethanol was omitted or the lysate was not completely transferred to the column.	Complete the RBCL and PBS steps without losing the pellet. Add 400 µL EcoSpin Lysis/Binding Buffer, mix well by pipetting, and pass the lysate through a 20-gauge (0.9 mm) needle at least 5-10 times or until homogeneous. Add 400 µL absolute ethanol, mix well, and transfer the full lysate to the column in 700 µL portions.
Lysate is viscous or not homogeneous	The recovered cell pellet was not sufficiently homogenized after addition of Lysis/Binding Buffer.	After adding 400 µL EcoSpin Lysis/Binding Buffer, mix by pipetting and pass the lysate through a 20-gauge (0.9 mm) needle attached to a sterile syringe at least 5-10 times or until a homogeneous lysate is obtained.
RNA integrity is poor / RNase inactivation may be insufficient	The sample has high RNase activity or handling before lysis was prolonged.	Proceed promptly from recovered cells to lysis. EcoSpin Lysis/Binding Buffer inactivates RNases; when additional RNase control is required, add 4 µL beta-mercaptoethanol to each 400 µL Lysis/Binding Buffer as described in the protocol.
Column flow is slow or sample remains on the membrane	The lysate was not fully homogenized, or more than 700 µL was loaded onto the column at once.	Ensure the lysate is homogeneous before adding ethanol. Load 700 µL per centrifugation, spin for 30 sec at maximum speed at room temperature, and repeat the loading step as necessary until the complete lysate has been processed.
Genomic DNA contamination affects sensitive downstream applications	Residual genomic DNA remained after the standard purification workflow.	Use the optional On-Column DNA Removal procedure when maximum removal of residual DNA is required for sensitive downstream applications.

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Problem	Most likely cause	Recommended action
Poor purity / downstream inhibition	Wash Buffer 2 was not prepared with ethanol, or the complete wash and final 2 min centrifugation sequence was not followed.	Before first use, add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate. Wash with 400 µL Wash Buffer 1 and 500 µL Wash Buffer 2, then add 200 µL Wash Buffer 2 and centrifuge at maximum speed for the full 2 min to remove residual wash buffer.
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. Use the specified 500 µL and 200 µL Wash Buffer 2 steps.
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 1.5 mL tube before elution.
Low RNA concentration in the eluate	The elution volume was unnecessarily large, Elution Buffer was not applied to the membrane center, or the 1 min incubation was skipped.	Apply 30-50 µL EcoSpin Elution Buffer directly to the center of the membrane, incubate for 1 min at room temperature, and centrifuge for 30 sec at maximum speed.
No or very little eluate recovered	Elution Buffer did not contact the membrane adequately, the incubation time was insufficient, or 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 1 min at room temperature, and centrifuge for 30 sec at maximum speed.
Results remain inconsistent despite correct reagent volumes	One or more RBCL, centrifugation, homogenization, wash, or elution steps differed from the protocol.	Repeat the workflow with the specified 1:9 RBCL dilution, approximately 1:6 blood-to-buffer ratio, 10 min and 5 min RBCL incubations, 1500 rpm pellet steps, 20-gauge needle homogenization, 400 µL ethanol addition, maximum-speed column spins, and 30-50 µL elution.

Still having trouble? Re-check the 1:9 RBCL dilution, room-temperature blood and working solution, approximately 1:6 blood-to-buffer ratio, 1500 rpm pellet steps, gentle supernatant removal, 20-gauge needle homogenization, 400 µL ethanol addition, 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 blood volume, anticoagulant, RBCL preparation details, pellet appearance, homogenization method, centrifugation conditions, and observed RNA yield or downstream issue.