

ClearBand 1x Red Blood Cell Lysis Buffer

Troubleshooting Guide

100 mL, 500 mL

Cat No: RBCLB-100, RBCLB-500

Use this guide for common problems encountered during red blood cell lysis and leukocyte recovery. For best results, follow the sample temperature, buffer-to-blood ratio, gentle mixing, incubation, centrifugation, and pellet-handling conditions in the product protocol.

Before You Start - Quick Checks

1. Warm the ready-to-use 1x lysis buffer to room temperature before use.
2. Use fresh whole blood at room temperature, collected in a tube containing EDTA or another anticoagulant.
3. Use approximately 1 part blood to 6 parts lysis buffer.
4. Mix by gentle inversion only and keep the sample protected from light during incubation. Do not vortex.
5. Use the specified centrifugation conditions: 1500 rpm for 5 min after the first lysis and 1500 rpm for 3 min after the second lysis.

Problem	Most likely cause	Recommended action
Incomplete red blood cell lysis / red cell contamination remains	Buffer or blood not at room temperature; incorrect blood-to-buffer ratio; insufficient mixing or incubation.	Warm the 1x buffer to room temperature before use and use fresh blood at room temperature. Maintain approximately a 1:6 blood-to-buffer ratio. Mix by gentle inversion and incubate for the full 10 min at room temperature, protected from light, with periodic hand inversion.
Pellet remains visibly red after first centrifugation	Residual erythrocytes remain after the first lysis step.	Carefully remove the clear red supernatant without disturbing the pellet. Gently resuspend the pellet in 1 mL 1x lysis buffer, incubate for 5 min at room temperature protected from light, then centrifuge at 1500 rpm for 3 min.
Low leukocyte recovery / small or lost pellet	Cell pellet disturbed or aspirated while removing supernatant; excessive handling during resuspension.	After each centrifugation, remove the supernatant carefully without disturbing the cell pellet. Resuspend gently and avoid vortexing. When transferring the pellet after the first centrifugation, use the protocol-specified 1 mL lysis buffer volume.
Cell clumping / difficult pellet resuspension	Pellet handled too aggressively or not resuspended gently and uniformly.	Resuspend the pellet gently in the specified buffer volume. Do not vortex. After the second lysis step, wash/resuspend the pellet in 1 mL PBS before proceeding to the final application buffer.
Variable results between samples	Differences in sample freshness, sample/buffer temperature, anticoagulant use, buffer ratio, incubation, or mixing.	Standardize the workflow: use fresh whole blood, bring both sample and buffer to room temperature, use anticoagulated blood, maintain the 1:6 ratio, keep incubation times consistent, and mix by gentle inversion at regular intervals.
Poor downstream performance after lysis	Residual lysis buffer or incomplete final wash before downstream processing.	After the second lysis and centrifugation, gently resuspend the pellet in 1 mL Phosphate Buffered Saline and pellet the cells as directed. Then resuspend the pellet in the appropriate downstream buffer before proceeding.
Excessive cell disturbance during processing	Vortexing or harsh mixing during lysis/resuspension steps.	Use gentle inversion by hand during the 10 min lysis and gentle resuspension for pellet handling. The protocol specifically instructs not to vortex.

Still having trouble? Repeat the procedure using a fresh sample and confirm that the buffer and blood were at room temperature, the 1:6 ratio was used, incubation times were followed, and pellets were handled gently. If the problem persists, contact EcoTech Biotechnology technical support with the sample volume, anticoagulant, buffer ratio, incubation times, centrifugation conditions, and observed pellet appearance.