

ClearBand 10x Red Blood Cell Lysis Buffer

Troubleshooting Guide

100 mL

Cat No: RBCLB-10x

Use this guide for common problems encountered while preparing the 1x working solution and during red blood cell lysis and leukocyte recovery. For best results, follow the dilution, pH, sample temperature, buffer-to-blood ratio, gentle mixing, incubation, centrifugation, and pellet-handling conditions in the product protocol.

Before You Start - Quick Checks

1. Prepare the 1x working solution just prior to use: dilute 1 part 10x buffer with 9 parts room-temperature double-distilled water.
2. Confirm the diluted 1x solution is at pH 7.1-7.4; adjust the pH if necessary, then warm the working solution to room temperature.
3. Use fresh whole blood at room temperature, collected in a tube containing EDTA or another anticoagulant.
4. Use approximately 1 part blood to 6 parts 1x lysis buffer. Mix by gentle inversion only and do not vortex.
5. Follow the specified incubation and centrifugation steps and remove supernatants carefully without disturbing the cell pellet.

Problem	Most likely cause	Recommended action
Incomplete red blood cell lysis / red cell contamination remains	Incorrect 10x-to-1x dilution; diluted buffer pH outside 7.1-7.4; sample or buffer not at room temperature; incorrect blood-to-buffer ratio; insufficient mixing or incubation.	Prepare fresh 1x buffer using 1 part 10x buffer + 9 parts room-temperature double-distilled water. Confirm pH 7.1-7.4 and warm to room temperature. Use 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.
Working solution gives variable results	Working solution prepared inconsistently, incorrect dilution ratio, or pH not checked after dilution.	Prepare the 1x working solution just prior to use. Use an accurate 1:9 dilution with room-temperature double-distilled water and verify that the diluted solution is within pH 7.1-7.4 before use.
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 diluted 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; harsh handling during pellet resuspension.	After centrifugation, remove supernatant carefully without disturbing the cell pellet. Resuspend gently and do not vortex. Follow the protocol-specified centrifugation conditions and buffer volumes.
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, gently resuspend the pellet in 1 mL PBS and pellet the cells before proceeding.
Variable results between blood samples	Differences in sample freshness, temperature, anticoagulant use, working-solution preparation, buffer ratio, incubation, or mixing.	Standardize the workflow: use fresh anticoagulated blood at room temperature, freshly prepared 1x buffer at pH 7.1-7.4, a consistent 1:6 ratio, fixed incubation times, and gentle inversion at regular intervals.
Poor downstream performance after lysis	Residual lysis buffer or incomplete final PBS 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.

Still having trouble? Prepare a fresh 1x working solution and verify the 1:9 dilution, pH 7.1-7.4, room-temperature sample/buffer conditions, 1:6 blood-to-buffer ratio, incubation times, and gentle pellet handling. If the problem persists, contact EcoTech Biotechnology technical support with the sample volume, anticoagulant, dilution and pH details, incubation times, centrifugation conditions, and observed pellet appearance.