

ClearBand Lyse & Load Troubleshooting Guide

5x1 ml

Cat No: LL5ML

Use this guide for common combined lysis and SDS-PAGE sample-preparation problems with ClearBand Lyse&Load.; The reagent combines RIPA Lysis Buffer and Laemmli Sample Buffer in one solution for direct protein sample preparation prior to SDS-PAGE.

Before You Start - Quick Checks

1. Store at room temperature.
2. Add 25 µL beta-mercaptoethanol per 1 mL Lyse&Load; before use.
3. The reagent does not include protease or phosphatase inhibitors; add them just before use when needed.
4. For 1×10^6 cells, use 0.1 mL Lyse&Load; homogenize as specified, keep on ice for 5 minutes, then boil for 5 minutes.

Problem	Most likely cause	Recommended action
Low protein release from the cell pellet	The lysis volume or disruption steps may be insufficient.	For 1×10^6 cells use 0.1 mL Lyse&Load; vortex thoroughly, and use 2-3 snap freeze-thaw cycles when higher yield is needed.
Lysate is highly viscous or difficult to load	The homogenization step may be incomplete.	Pass the lysate through a 20-gauge needle 5-10 times or until a homogeneous lysate is obtained.
Protein degradation is observed	The reagent does not include protease inhibitors.	Add a suitable protease inhibitor to Lyse&Load; just prior to use when preservation against proteolysis is required.
Phosphorylation is not preserved	The reagent does not include phosphatase inhibitors.	Add an appropriate phosphatase inhibitor just prior to use when phosphorylation status must be maintained.
Reduction is incomplete	Beta-mercaptoethanol may have been omitted.	Add 25 µL beta-mercaptoethanol per 1 mL Lyse&Load; before sample preparation.
Western blot bands improve only after extra clarification	Cell-type or setup-specific debris may remain in the lysate.	Use the optional 4°C, 13,000 rpm, 10-minute centrifugation and transfer the supernatant before boiling when clearer bands are needed.
Sample preparation varies between experiments	Incubation and handling steps may not be standardized.	Use the 30-second room-temperature lysis period, homogenization, 5-minute ice step, and 5-minute boiling step consistently.
Residual culture medium affects lysis	The starting cell pellet may not have been washed as specified.	Wash the 1×10^6 cells in chilled PBS and remove the supernatant before adding Lyse&Load;.

Still having trouble? Recheck the 25 µL/mL beta-mercaptoethanol addition, inhibitor requirements, 0.1 mL per 1×10^6 cells, homogenization, optional clarification, and 5-minute boiling step.