

ClearBand RIPA Lysis Buffer

Troubleshooting Guide

100 ml

Cat No: RIPA-100

Use this guide for common cell- and tissue-lysis problems with ClearBand RIPA Lysis Buffer. The ready-to-use buffer is intended for extraction of cytoplasmic, membrane, and nuclear proteins and is compatible with protein assays, immunoassays, reporter assays, and protein purification workflows.

Before You Start - Quick Checks

1. Store at 4°C and warm the buffer to room temperature before use.
2. The buffer does not include protease or phosphatase inhibitors; add them just prior to use when required.
3. Keep samples cold after lysis and centrifuge at 4°C.
4. For cells, the datasheet uses 0.1 mL per 1×10^6 cells; for tissue, use thorough liquid-nitrogen grinding or rotor-stator homogenization.

Problem	Most likely cause	Recommended action
Low protein yield from cultured cells	The cell pellet may not be fully lysed or the buffer volume may be insufficient.	Use 0.1 mL RIPA per 1×10^6 cells, vortex thoroughly, and use 2-3 snap freeze-thaw cycles when increased yield is needed.
Low protein yield from tissue	Tissue disruption may be incomplete.	Use either thorough liquid-nitrogen grinding followed by immediate RIPA addition or vigorous rotor-stator homogenization at 15,000 rpm for 30 seconds.
Protein degradation is observed	Protease inhibitors may have been omitted.	Add protease inhibitors to RIPA just prior to use when proteolysis must be minimized.
Phosphorylation signal is lost	Phosphatase inhibitors may have been omitted.	Add phosphatase inhibitors just prior to use when phosphorylation status must be preserved.
Precipitate appears after adding PMSF	PMSF was added while the RIPA buffer was cold.	Warm ClearBand RIPA Lysis Buffer to room temperature before PMSF addition, as specified in the datasheet.
Lysate remains turbid after lysis	Insoluble debris may not have been removed.	After the ice incubation, centrifuge at 4°C and 13,000 rpm for 10 minutes and transfer the supernatant to a new tube.

Table continues on next page.

ClearBand RIPA Lysis Buffer Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Cell lysis varies between runs	The freeze-thaw, vortex, or ice-incubation steps may differ.	Standardize thorough vortexing, 2-3 freeze-thaw cycles when used, and the 10-15 minute ice incubation.
Tissue warms during grinding	The sample may thaw before buffer addition.	When using liquid nitrogen, grind thoroughly and add RIPA immediately after nitrogen evaporates without allowing the tissue to thaw.
Rotor-stator tissue lysate has poor yield	Homogenization intensity or duration may be insufficient.	Use the datasheet condition of 15,000 rpm for 30 seconds and ensure tissue is fully disrupted.
Clarified supernatant is contaminated with debris	The pellet may have been disturbed during transfer.	Transfer only the clear supernatant after the 4°C centrifugation step.
Downstream SDS-PAGE sample preparation is inconsistent	Laemmli addition or boiling may not be standardized.	After quantification, add ClearBand Laemmli Sample Buffer and boil for 5 minutes as in the supplied workflow.
Buffer was used directly from 4°C	The datasheet calls for warming before use and warns about PMSF precipitation in cold buffer.	Warm the required RIPA volume to room temperature before the lysis setup, then keep the sample cold after lysis.

Still having trouble? Recheck 4°C storage, warming before use, inhibitor addition, sample-to-buffer amount, complete mechanical disruption, 10-15 minutes on ice, and 4°C clarification before changing downstream detection conditions.