

ClearBand RIPA Lysis Buffer, Complete System

Troubleshooting Guide

50 ml RIPA + 0.5 ml 100x Protease Inhibitor Cocktail + 0.5 ml 100x PMSF

Cat No: RIPA50CS

Use this guide for common protein-extraction problems with ClearBand RIPA Lysis Buffer, Complete System. The kit combines ready-to-use RIPA with 100x Protease Inhibitor Cocktail and 100x PMSF for protected extraction from cultured cells and tissue.

Before You Start - Quick Checks

1. Store RIPA at 4°C and the 100x Protease Inhibitor Cocktail and 100x PMSF at -20°C.
2. For each 1 mL RIPA, add 10 µL 100x Protease Inhibitor Cocktail and 10 µL 100x PMSF.
3. Prepare only the working volume needed when possible, keep it cold, and add PMSF shortly before use.
4. Keep samples cold during extraction and clarify lysates at 12,000-14,000 x g for 10 minutes at 4°C.

Problem	Most likely cause	Recommended action
Protein degradation is observed	Protease Inhibitor Cocktail or PMSF may have been omitted, added at the wrong ratio, or added too early.	For each 1 mL RIPA add 10 µL of each 100x inhibitor and prepare the working buffer shortly before use.
Working buffer was prepared with the wrong inhibitor ratio	The 100x stocks may have been treated as a different concentration.	Use 10 µL Protease Inhibitor Cocktail plus 10 µL PMSF per 1 mL RIPA.
PMSF protection is inconsistent	PMSF is unstable in aqueous solution and may have been added too far in advance.	Add PMSF to the working buffer shortly before use and prepare only the amount needed when possible.
Low yield from adherent cells	The working buffer volume or scraping/lysis steps may be insufficient.	Use about 100-300 µL per well of a 6-well plate, scrape thoroughly, and complete the freeze-thaw and ice-incubation steps.
Low yield from suspension cells	The pellet may not be fully resuspended or the working volume may be inappropriate.	Use about 100-500 µL per 1x10 ⁶ to 5x10 ⁶ cells and pipette thoroughly before the freeze-thaw step.
Low yield from tissue	The tissue-to-buffer ratio or homogenization may be inadequate.	Start with about 500-1000 µL per 100 mg tissue and use complete liquid-nitrogen grinding or rotor-stator homogenization.
Lysate is highly viscous	Genomic DNA may have been released into the lysate.	Use brief sonication or repeated pipetting to reduce viscosity, as noted in the datasheet.

Table continues on next page.

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Problem	Most likely cause	Recommended action
Clarified lysate still contains debris	Centrifugation may be insufficient or the pellet may have been disturbed.	Centrifuge at 12,000-14,000 x g for 10 minutes at 4°C and carefully transfer only the clear supernatant.
Protein yield varies between samples	Buffer volume, sample amount, or lysis intensity may not be standardized.	Keep the sample-to-buffer ratio, freeze-thaw treatment, ice incubation, and mixing consistent between samples.
Tissue sample warms during preparation	Cold handling may have been interrupted.	Keep tissue and reagents cold, prevent thawing during liquid-nitrogen grinding, and use pre-cooled tubes.
Prepared working RIPA was stored incorrectly	Supplemented RIPA has different storage guidance from unsupplemented RIPA.	Store base RIPA at 4°C, inhibitor stocks at -20°C, and supplemented RIPA at -20°C if it must be stored.
Lysate loses quality during storage	Repeated freeze-thaw of prepared lysate may be occurring.	Aliquot lysates; keep on ice for short-term handling and store at -20°C or preferably -80°C for longer storage.
Some targets extract poorly	Lysis efficiency may vary with sample type and target localization.	Keep the core protocol constant first, then optimize lysis conditions for the target only if needed.
Downstream SDS-PAGE preparation is inconsistent	Clarified lysates may not be quantified and processed consistently.	After clarification, quantify the supernatant, add ClearBand Laemmli Sample Buffer, and boil for 5 minutes.
Inhibitors were added to the full bottle immediately	The datasheet recommends preparing only the amount needed when possible.	Prepare a smaller working volume with fresh inhibitor additions to preserve the stock components and PMSF effectiveness.

Still having trouble? Recheck component-specific storage, the 10 µL + 10 µL inhibitor additions per 1 mL RIPA, cold handling, sample-to-buffer ratio, clarification, viscosity control, and lysate storage before changing the downstream assay.