

ClearBand 10x RIPA Lysis Buffer

Troubleshooting Guide

100 ml

Cat No: RIPA10x

Use this guide for common dilution, lysis, extraction, inhibitor, precipitation, and clarification problems with ClearBand 10x RIPA Lysis Buffer. The product is intended for cytoplasmic, membrane, and nuclear protein extraction and is compatible with Western blotting and immunoprecipitation.

Before You Start - Quick Checks

1. Dilute the 10x stock to 1x with ddH₂O before lysis.
2. The buffer does not contain protease or phosphatase inhibitors; add them just prior to use when required.
3. PMSF added to cold 1x RIPA may form precipitate.
4. For 1x10⁶ cells, the datasheet uses 0.1 ml 1x RIPA, followed by 10-15 min on ice and centrifugation at 4°C, 13,000 rpm for 10 min.
5. For tissue, use the datasheet grinding or rotor-stator homogenization workflow and add 500 uL 1x RIPA as described.

Problem	Most likely cause	Recommended action
Cell lysate yield is low	The 10x stock may not have been diluted to 1x correctly or cell disruption may be incomplete.	Verify the 10x-to-1x dilution. For cells, vortex thoroughly and use the datasheet 2-3 snap freeze-thaw cycles when increased yield is needed.
Tissue lysate yield is low	Tissue disruption may be incomplete or tissue may have thawed during the liquid-nitrogen workflow.	Follow one of the datasheet tissue-homogenization methods completely and add 500 uL 1x RIPA immediately after disruption.
Pellet remains after cell lysis	Cell disruption or solubilization may be incomplete.	Vortex thoroughly, use 2-3 snap freeze-thaw cycles when needed, then keep on ice for 10-15 min before clarification.
Lysate is cloudy after incubation	Insoluble material may not have been removed.	Centrifuge at 4°C and 13,000 rpm for 10 min, then transfer only the clarified supernatant.
Protein appears degraded	Protease inhibitors may not have been added when required.	The stock does not include protease inhibitors; add the required inhibitor(s) to 1x RIPA just prior to use according to the experimental plan.
Phosphorylation signal is lost	Phosphatase inhibitors may not have been included when phosphorylation status needed preservation.	The stock does not contain phosphatase inhibitors; add them just prior to use when the experiment requires preservation of phosphorylation.
Precipitate appears after PMSF addition	PMSF added to cold 1x RIPA can form precipitate, as noted in the datasheet.	Recognize this as a documented possibility and prepare inhibitor-supplemented buffer just prior to use; ensure the lysis mixture is handled consistently.

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Problem	Most likely cause	Recommended action
Cell lysis volume is inconsistent	The amount of 1x RIPA used per cell number may vary between samples.	For the datasheet reference condition, use 0.1 ml 1x RIPA for 1×10^6 cells and scale consistently when needed.
Sample warms during extraction	The workflow may not be maintaining the cold conditions specified for incubation and centrifugation.	Keep samples on ice for 10-15 min and clarify at 4°C as described.
Tissue sample thaws during grinding	The liquid-nitrogen grinding workflow may have been delayed.	Do not allow the tissue to thaw; after liquid nitrogen evaporates, immediately add 500 μ L 1x RIPA.
Rotor-stator tissue lysis is incomplete	Homogenization conditions may not match the datasheet procedure.	For the rotor-stator option, homogenize immediately at 15,000 rpm for 30 sec after adding 500 μ L 1x RIPA.
Downstream SDS-PAGE sample is inconsistent	The clarified supernatant may not have been quantified or processed consistently.	After centrifugation, transfer the supernatant, quantify it, add ClearBand Laemmli Sample Buffer, and boil for 5 min as specified.
Stock has been stored for an extended period	The stock may be beyond the stated stability window or precipitation may be present.	The datasheet states at least 6 months at room temperature and notes additional use after expiration only when no precipitation is observed; inspect the stock before use.
Working buffer was prepared with inhibitors long before use	The datasheet specifies adding protease/phosphatase inhibitors just prior to use.	Prepare inhibitor-supplemented 1x RIPA immediately before the lysis workflow.
Results differ between cell and tissue samples	The sample-disruption procedures are different for cells and tissues.	Use the specific cell-lysis or tissue-lysis workflow in the datasheet rather than applying one disruption method to both sample types.

Still having trouble? Recheck 10x-to-1x dilution, sample-specific disruption, 10-15 min on ice, cold clarification at 13,000 rpm for 10 min, and whether protease/phosphatase inhibitors were added just prior to use when required.