

ClearBand 100x Protease Inhibitor Cocktail

Troubleshooting Guide

1 ml, 5x1 ml

Cat No: PIC1, PIC5

Use this guide for common protein-protection problems with ClearBand 100x Protease Inhibitor Cocktail. The cocktail contains AEBSF, aprotinin, bestatin, E-64, leupeptin, and pepstatin A in DMSO. EDTA is supplied separately for metalloprotease inhibition when appropriate.

Before You Start - Quick Checks

1. Store at -20°C and thaw the cocktail on ice before use.
2. Immediately before cell lysis or tissue extraction, add the cocktail to the lysis or extraction buffer at 1:100 (v/v).
3. Decide separately whether EDTA is appropriate for the downstream workflow.
4. For IMAC or 2D gel workflows, account for the datasheet warning that EDTA must be removed before purification or analysis.

Problem	Most likely cause	Recommended action
Protein degradation is still visible	The cocktail may have been omitted, added after lysis/extraction, or used below 1:100.	Add the inhibitor to the lysis or extraction buffer immediately before cell lysis or tissue extraction at 1:100 (v/v).
Results vary between lysates	The inhibitor-to-sample ratio may differ between tubes.	Calculate the cocktail volume from the working lysis/extraction buffer volume and keep the 1:100 ratio constant across samples.
Metalloprotease activity remains a concern	The six-inhibitor cocktail does not itself provide the separately supplied EDTA component.	Evaluate whether EDTA should be added for metalloprotease inhibition in the specific experiment.
A divalent-cation-dependent protein loses activity after EDTA addition	EDTA chelates divalent cations and can affect activities of other proteins.	Repeat the workflow without EDTA or empirically determine whether EDTA is suitable for the protein and downstream assay.
IMAC purification performs poorly	EDTA can interfere with immobilized metal chelate affinity chromatography.	Remove EDTA by extensive dialysis or desalting before IMAC, as specified in the datasheet.
2D gel workflow is affected	EDTA carried into the downstream workflow may be incompatible with the intended analysis.	Remove EDTA by extensive dialysis or desalting before 2D gel electrophoresis.
Cocktail was thawed at room temperature	Handling did not follow the instruction to thaw on ice.	Thaw the product on ice and keep the inhibitor cold during sample preparation.
Phosphorylation is still lost even though proteolysis is controlled	Protease inhibition does not replace phosphatase inhibition.	Use an appropriate phosphatase inhibitor cocktail when preservation of phosphorylation is required.

Still having trouble? Confirm -20°C storage, thawing on ice, immediate 1:100 addition to the lysis/extraction buffer, and whether EDTA is compatible with the target protein and downstream method.