

ClearBand 100x Phosphatase Inhibitor Cocktail

Troubleshooting Guide

1 ml

Cat No: PHIC1

Use this guide for common handling and inhibition problems with ClearBand 100x Phosphatase Inhibitor Cocktail. The product is a five-inhibitor blend intended to suppress acid phosphatases, alkaline phosphatases, and protein tyrosine phosphatases during cell lysis and protein extraction.

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/extraction buffer at 1:100 (v/v).
3. Use the same inhibitor-to-buffer ratio across all samples for comparable phosphorylation preservation.
4. This is a phosphatase inhibitor cocktail; protease protection requires a separate strategy when needed.

Problem	Most likely cause	Recommended action
Loss of phosphorylation signal	The cocktail may have been omitted, added after lysis/extraction began, or used below the recommended 1:100 dilution.	Prepare a fresh sample with the cocktail added to the lysis/extraction buffer at 1:100 (v/v) immediately before cell lysis or tissue extraction. Keep the cocktail on ice during setup.
Phosphoprotein results vary between samples	The inhibitor-to-buffer ratio or the timing of inhibitor addition may not be consistent between samples.	Calculate the cocktail volume from the final lysis/extraction buffer volume and use the same 1:100 ratio and addition timing for every sample.
Inhibitor was added after lysis or extraction started	Endogenous phosphatases may have remained active during early sample handling.	Repeat sample preparation with inhibitor already present in the lysis/extraction buffer before it contacts the cells or tissue.
Cocktail was thawed on the bench	Handling did not follow the datasheet instruction to thaw on ice.	Thaw the product on ice before use and return it promptly to cold storage after the required volume is removed.
Only some phosphatase classes appear controlled	The experimental readout may involve phosphatase activity outside the inhibition profile or sample-specific conditions.	First confirm correct 1:100 use and prompt addition. The datasheet specifies broad inhibition of acid phosphatases, alkaline phosphatases, and protein tyrosine phosphatases.
Protein degradation is still observed	A phosphatase inhibitor cocktail does not replace protease inhibition.	Use an appropriate protease-protection strategy when proteolysis is also a concern.
Inhibition seems excessive in a phosphatase-dependent downstream assay	The cocktail is intentionally designed to suppress endogenous phosphatases.	Do not carry inhibitor into a downstream assay that requires phosphatase activity unless the workflow has been validated for that purpose.
Wrong stock volume was added	The 100x designation may have been interpreted incorrectly.	Use a 1:100 (v/v) addition; for example, add 10 µL cocktail to 990 µL lysis/extraction buffer for 1 mL final inhibited buffer.

Still having trouble? Recheck -20°C storage, thawing on ice, the 1:100 (v/v) addition, and that the inhibitor is added to the lysis/extraction buffer immediately before cell lysis or tissue extraction.