Store at 4°C

#5872

Protease/Phosphatase Inhibitor Cocktail (100X)

1 ml

www.cellsignal.com

Support: 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: When diluted in lysis buffer to a final concentration of 1X the Protease/Phosphatase Inhibitor Cocktail prevents protein degradation and dephosphorylation by endogenous proteases and phosphatases present in the whole cell extract. The 100X cocktail is a clear light yellow to light green liquid.

Background: Dynamic protein phosphorylation is a key cellular signaling mechanism by which a broad spectrum of cellular processes is regulated. In order to study the phosphorylation status of specific target proteins the phosphorylated residue of interest must remain intact. When cells are lysed to make whole cell extracts, a loss of normal cellular signaling regulation occurs, and phosphatases within the cell extract are free to dephosphorylate proteins in an uncontrolled manner. The addition of phosphatase inhibitors to the cell lysis buffer aids in the preservation of phosphorylated residues at the time of cell disruption.

This same loss of normal cellular control when generating whole cell extracts also leads to uncontrolled degradation of proteins by endogenous proteases. The addition of protease inhibitors to the cell lysis buffer aids in the preservation of target proteins in the cell extract.

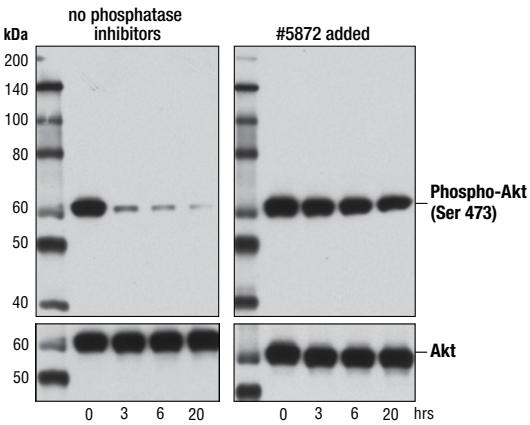
Directions for Use:
1. Briefly vortex the Protease/Phosphatase Inhibitor Cocktail (100X) before use.

2. Just prior to lysing cells, dilute the cocktail 1:100 in desired lysis buffer to obtain a 1X working concentration.

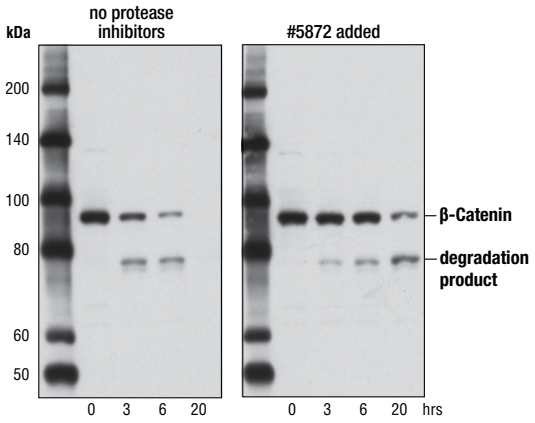
Solutions and Reagents: The Protease/Phosphatase Inhibitor Cocktail (100X) is composed of a proprietary mix of Aprotinin, Bestatin, E64, and Leupeptin to promote broad spectrum protection against endogenous proteases and sodium fluoride, sodium pyrophosphate, β-glycerophosphate, and sodium orthovanadate to promote broad spectrum protection against endogenous serine/threonine and tyrosine phosphatases. The cocktail does not contain EDTA (a metalloprotease inhibitor) which can be incompatible with some downstream applications (i.e. protein assays, 2D electrophoresis, etc.). If EDTA is desired as a protease inhibitor it can be added to the cell lysis buffer at a final working concentration of 5mM.

Storage: Store the undiluted 100X cocktail at 4°C. *Do not freeze.* This product is stable for 12 months.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.



Western blot analysis of extracts from NIH/3T3 cells, serum-starved overnight and treated with hPDGF-BB #8912 (100ng/ml, 5min), prepared in lysis buffer in the absence of phosphatase inhibitors (left) or with Protease/Phosphatase Inhibitor Cocktail (100X) #5872 added (right), and incubated at 37°C for the indicated time points, using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 (upper) or Akt (pan) (C67E7) Rabbit mAb #4691 (lower). In the absence of phophatase inhibitors, phospho-Akt signal drops significantly after time point 0, demonstrating rapid loss of phosphorylation at later time points. In the presence of the phosphatase inhibitor cocktail, the phospho-Akt signal is preserved through all time points monitored.



Western blot analysis of extracts from NIH/3T3 cells, prepared in lysis buffer in the absence of protease inhibitors (left) or with Protease/Phosphatase Inhibitor Cocktail (100X) #5872 added (right), and incubated at 37°C for the indicated time points, using β-Catenin (D10A8) XP® Rabbit mAb #8480. In the absence of protease inhibitors, β-Catenin signal fades within 3 hr after harvest, indicating protein degradation. In the presence of the protease inhibitor cocktail, the β-Catenin degradation is slowed significantly and signal is still present at 20 hr following harvest.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.