

## Phosphatase Inhibitor Cocktail II

### Overview

<b>Product Name</b>	<b>Phosphatase Inhibitor Cocktail II</b>
<b>Catalog Number</b>	WAR1120
<b>Physical State</b>	White lyophilized powder
<b>Pack Size</b>	80mg (sufficient for 100mL of extract)
<b>Content</b>	Proprietary mix of: Sodium fluoride, Sodium orthovanadium, Imidazole, Sodium molybdate, Sodium tartaric
<b>Recommended working concentration</b>	Reconstitute Phosphatase Inhibitor Cocktail II with 1mL of water.
<b>Storage</b>	Upon receipt store at -20°C. It is stable for one year. Product is shipped on ice.

### Biochemical Information

<b>Inhibitor</b>	<b>Inhibition Specificity</b>
<b>Sodium Flouride</b>	Acid Phosphatase, Serine phosphatases, Threonine phosphatases
<b>Sodium Orthovanadium</b>	Alkaline phosphatases, Tyrosine phosphatase
<b>Imidazole</b>	Histidine phosphatase
<b>Sodium molybdate</b>	Acid phosphatase
<b>Sodium tartrate</b>	Acid phosphatase

### Assay Principle

Crude cell or tissue extracts contain a number of endogenous enzymes, such as proteases and phosphatases, which are capable of modifying the proteins present in the extracts. Inhibitors of these enzymes should be added to improve the yield of native proteins. Phosphatase inhibitor cocktail II protect phosphoproteins from tyrosine protein phosphatases, acid and alkaline phosphatases. The cocktail contains a mixture of five inhibitors, including sodium fluoride, sodium orthovanadium, imidazole, sodium molybdate, and sodium tartaric. It is suitable for use with many kinds of cell and tissue extract, including primary cells, mammalian cells, animal tissue, plant tissue, yeast or bacterial cells.

### Properties

<b>Compatibility with reagents</b>	<b>Fully compatible with cell lysis buffers and Broad Spectrum Protease Inhibitor Cocktail</b>
<b>Compatibility with assays</b>	Compatible with IEF/2D studies; MS-compatible: not contain AEBSEF; Compatible with immobilized metal chelate affinity chromatography and 2D gel electrophoresis inhibitor components dialysis removal or desalting;
<b>Reagent Type</b>	Western Blotting related reagent; Inhibitors
<b>Usage</b>	To preserve phosphorylation state and protein functionality following cell lysis
<b>Target Specificity</b>	Tyrosine protein phosphatases, acid and alkaline phosphatases

<b>Target Sample</b>	Cell lysis extracts
<b>Description</b>	Phosphatase Inhibitor Cocktail II is a Western blot related inhibitor that is to be added to cell lysis buffer to protect native phosphoproteins from dephosphorylation during proteins purification and sample preparation used in WB, Co-IP and IP.
<b>Application</b>	Animal tissue and cell extract with detergent.

## Background

Phosphatase inhibitors are used when phosphorylation (activation) states of target proteins need to be studied and the phosphorylated residues of interest must remain intact. They are chemicals that aid in the extraction of intact proteins in their native modification state by inhibiting endogenous phosphatases that would otherwise dephosphorylate the proteins present in cell lysates and tissue extracts. Phosphatase Inhibitor Cocktail II contains individual components with specific inhibitory properties to provide an all-around protection of the protein phosphorylation state. Dynamic protein phosphorylation is a key cellular signaling mechanism for cell processes regulation. When tissues are lysed to make whole cell extracts, the loss of natural compartmentalization causes normal regulation of cellular signaling to get distorted, and resident cell phosphatases within the cell extract are free to disorderly dephosphorylate proteins. The usual consequence of this unregulated state is biologically meaningless representation of protein activities (i.e. phosphorylation status) and false negative staining in anti-phosphoprotein immunostaining analyses. The addition of phosphatase inhibitors to the cell lysis buffer aids in the preservation of phosphorylated residues at the time of cell disruption.

## Features

- Dialyzable—inhibitors can be removed by dialysis, before performing assays known to be incompatible with phosphatase inhibitors.
- Supplied as a 100X concentrate—effective as 1X. For samples with high level of phosphatase may need 2-3X.
- Detergent compatible—can be used with lysis buffers and do not cross react with detergents in the buffers.
- Protein Assay Compatible—works with BCA and commassie blue assays.

## Usage and Handling

The product is supplied as white lyophilized powders. Since phosphatase levels may vary among cell and tissue types, it may be necessary to increase the concentration of inhibitors.

## Protocol

1. Reconstitute Phosphatase Inhibitor Cocktail II in 1ml of double distilled water.
2. Add cocktail solution to extraction buffer in 1:100. (e.g., Add 10  $\mu$ L of cocktail solution to 1mL extraction buffer).
3. Proceed to extract protein fractions.

**Notes:** Aliquot and store reconstituted phosphatase inhibitor cocktail solution at -20°C.