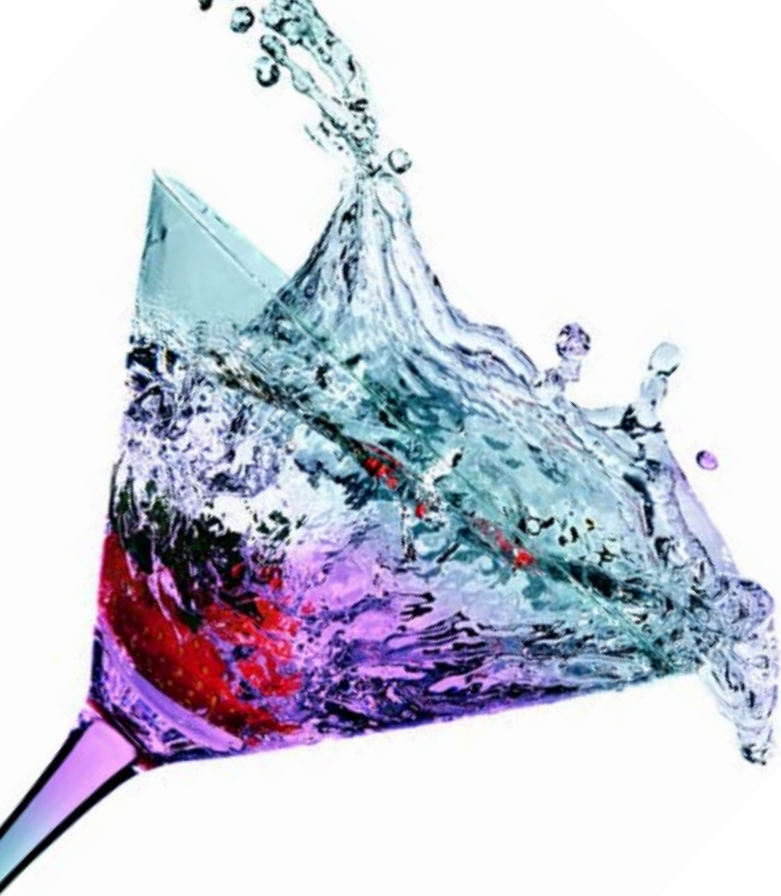


# Protease and Phosphatase Inhibitor Cocktails

Application & Product Selection Guide



What's the recipe for successful proteomics research?

Take one of our pre-mixed protease or phosphatase inhibitor cocktails, add your sample - and stir! Easy to use, consistent blend, and a no-waste format ...the perfect mix!

That's what's in it for you. EMD Chemicals

## Protease Inhibitor Cocktails and Inhibitor Sets

Protease inhibitor cocktails and highly specific inhibitors to proteases are important tools for scientists engaged in a variety of proteomic studies including protein expression and characterization, biomarker discovery, mapping of post-translational modifications, protein expression profiling, and the quantitative measurement of proteins. The addition of a protease inhibitor cocktail or protease inhibitor ensures the integrity of proteins for downstream analysis and further characterization of samples.

During protein expression and isolation, endogenous proteases rapidly begin to degrade protein samples following cell lysis or tissue disaggregation. The resulting proteolysis

can drastically reduce the quality and quantity of protein samples required for characterization and analysis. In order to overcome this problem, complex protein solutions are treated with a mixture or cocktail of protease inhibitors, which help preserve the activity and nature of proteins. By utilizing a specific combination of protease inhibitors, preparative protein samples may be protected from the most common proteases including serine proteases, metalloproteases, cysteine proteases, aminopeptidases, and aspartic proteases.

Using a combination of synthetic, recombinant, and naturally occurring protease inhibitors, we have developed a panel of 12 application specific protease inhibitor cocktails. These cocktails are formulated using reagents of the highest quality and biological activity. A convenient summary about the use of these cocktails is outlined on page 3.

### Key Features:

**Convenient** | Flexible protocol. Ready-to-use formulations.

**Consistent** | High quality ensures reproducibility and excellent inhibition over a wide range of protease classes.

**Flexible** | A comprehensive selection of specific cocktail formulations designed to inhibit proteolytic activity from most tissue or cell type extracts, including mammalian, bacterial, yeast, fungal, and plant cells.

**Application Specific** | Available without EDTA for purification schemes involving metal ion chelating chromatography or analysis using 2-D gel electrophoresis. Option of new protease inhibitor cocktail formulations using recombinant aprotinin for applications that require the use of animal-free reagents.

# Selection Guide for the Use of Specialized Protease Inhibitor Cocktails

Product	Cat No.	Recommended Application
Protease Inhibitor Cocktail Set I	539131	General use
Protease Inhibitor Cocktail Set I, Animal-Free	535142	General use and for applications that require the use of animal-free reagents
Protease Inhibitor Cocktail Set II	539132	Bacterial cell extracts (except those intended for metal chelation chromatography)
Protease Inhibitor Cocktail Set III, EDTA-Free	539134	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis
Protease Inhibitor Cocktail Set III, Animal-Free	535140	Mammalian cells and tissue extracts and for applications that require the use of animal-free reagents
Protease Inhibitor Cocktail Set IV	539136	Fungal and yeast cell extracts
Protease Inhibitor Cocktail Set V, EDTA Free	539137	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis
Protease Inhibitor Cocktail Set V, Animal-Free	535141	Mammalian cells and tissue extracts and for applications that require the use of animal-free reagents
Protease Inhibitor Cocktail Set VI	539133	Plant cell extracts
Protease Inhibitor Cocktail Set VII	539138	Proteins containing His•Tag® sequences
Protease Inhibitor Cocktail Set VIII	539129	Broad range cysteine protease inhibition
Serine Protease Inhibitor Cocktail Set I	565000	Broad range serine protease inhibition
Protease Inhibitor Set	539128	Screening for suitable inhibitor
Protease Arrest™ Reagent	539128	Broad range of serine, cysteine, and calpain protease inhibition

## In This Guide:

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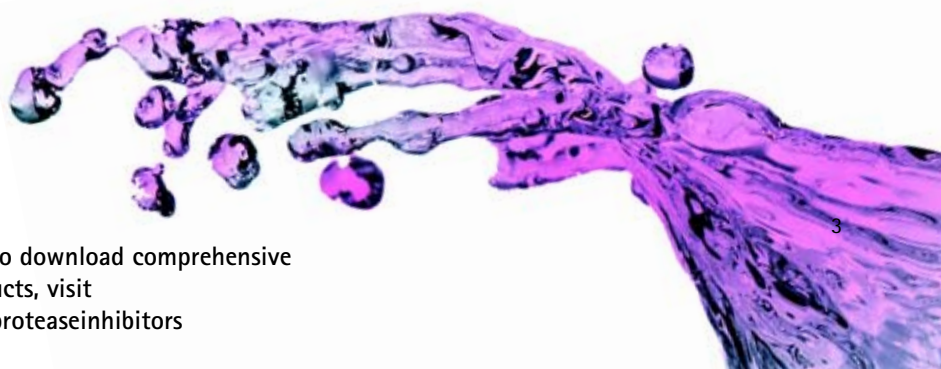
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### Protease Inhibitor Cocktail Set I

Cat No. 539131

Form: Lyophilized

A cocktail containing five protease inhibitors that will inhibit a broad range of proteases. Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. When diluted to 1X stock solution, each vial contains the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor	M.W.	1X Concentration	Target Proteases
AEBSF, Hydrochloride	239.5	500 μM	Serine Proteases
Aprotinin, Bovine Lung	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 μM	Cysteine Proteases
EDTA, Disodium	372.2	500 μM	Metalloproteases
Leupeptin, Hemisulfate	475.6	1 μM	Cysteine Proteases and Trypsin-like Proteases

### Protease Inhibitor Cocktail Set I, Animal-Free

Cat No. 535142

Form: Lyophilized

This animal-free formulation includes the same five protease inhibitors as the original version (Cat. No. 539131), but substitutes Aprotinin, Bovine Lung with a recombinant Aprotinin. Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. Dilute to 1X before use.

Protease Inhibitor	M.W.	1X Concentration	Target Proteases
AEBSF, Hydrochloride	239.5	500 μM	Serine Proteases
Aprotinin, Recombinant	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 μM	Cysteine Proteases
EDTA, Disodium	372.2	500 μM	Metalloproteases
Leupeptin, Hemisulfate	475.6	1 μM	Cysteine Proteases and Trypsin-like Proteases

### Protease Inhibitor Cocktail Set II

Cat No. 539132

Form: Lyophilized

This cocktail is recommended for use with bacterial cell extracts (except those intended for metal chelation chromatography). Cocktail contains five protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, serine, and metalloproteases as well as aminopeptidases. Reconstitute each vial with 1 ml DMSO and 4 ml H<sub>2</sub>O to obtain a 5 ml stock solution. When reconstituted, each vial will contain the concentrations of inhibitors shown in the table on the right. One set is sufficient for about 20 g cells. Note: 1 ml of DMSO included per set.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	20 mM	Serine Proteases
Bestatin	308.4	1.7 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	200 μM	Cysteine Proteases
EDTA, Disodium	372.2	85 mM	Metalloproteases
Pepstatin A	685.9	2 mM	Aspartic Proteases

### Protease Inhibitor Cocktail Set III, EDTA-Free

Cat No. 539134

Form: Liquid

This cocktail is recommended for use with mammalian cells and tissue extracts and for bacterial cell extracts being used for metal ion chelation chromatography. Cocktail contains six protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table on the right. One ml is sufficient for about 20 g tissue.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
Aprotinin, Bovine Lung	6512	80 μM	Serine Proteases and Esterases
Bestatin	308.4	5 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	2 mM	Cysteine Proteases and Trypsin-like Proteases
Pepstatin A	685.9	1 mM	Aspartic Proteases

### Protease Inhibitor Cocktail Set III, Animal-Free

Cat No. 535140

Form: Liquid

This animal-free formulation includes the same six protease inhibitors as the original version (Cat. No. 539134), but substitutes Aprotinin, Bovine Lung with a recombinant Aprotinin. Each vial contains the concentrations of inhibitors shown in the table on the right (in 1 ml DMSO). One ml is sufficient for about 20 g tissue.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
Aprotinin, Recombinant	6512	80 $\mu$ M	Serine Proteases and Esterases
Bestatin	308.4	5 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	2 mM	Cysteine Proteases and Trypsin-like Proteases
Pepstatin A	685.9	1 mM	Aspartic Proteases

### Protease Inhibitor Cocktail Set IV

Cat No. 539136

Form: Liquid

This cocktail is recommended for fungal and yeast cell extracts. Cocktail contains four protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of aspartic, cysteine, metallo-, and serine proteases. Each vial contains the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Proteases
Pepstatin A	685.9	2 mM	Aspartic Proteases
<i>o</i> -Phenanthroline	198.2	500 mM	Metalloproteases

### Protease Inhibitor Cocktail Set V, EDTA-Free

Cat No. 539137

Form: Lyophilized

An EDTA-free cocktail containing four protease inhibitors for the inhibition of serine and cysteine proteases, but not metalloproteases. Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. When diluted to 1X stock solution, the set will contain the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor	M.W.	1X Concentration	Target Proteases
AEBSF, Hydrochloride	239.5	500 $\mu$ M	Serine Proteases
Aprotinin, Bovine Lung	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 $\mu$ M	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	1 $\mu$ M	Cysteine Proteases and Trypsin-like Proteases

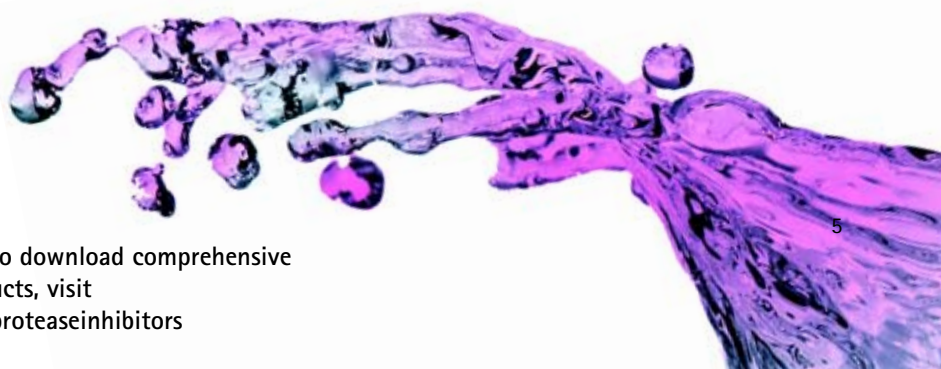
### Protease Inhibitor Cocktail Set V, Animal-Free

Cat No. 535141

Form: Lyophilized

This animal-free formulation includes the same four protease inhibitors as the original version (Cat. No. 539137), but substitutes Aprotinin, Bovine Lung with a recombinant Aprotinin. Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. Dilute to 1X before use.

Protease Inhibitor	M.W.	1X Concentration	Target Proteases
AEBSF, Hydrochloride	239.5	500 $\mu$ M	Serine Proteases
Aprotinin, Recombinant	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 $\mu$ M	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	1 $\mu$ M	Cysteine Proteases and Trypsin-like Proteases



For our current pricing or to download comprehensive information on these products, visit [www.emdbiosciences.com/proteaseinhibitors](http://www.emdbiosciences.com/proteaseinhibitors)



### Protease Inhibitor Cocktail Set VI

Cat No. 539133

Form: Liquid

This cocktail is recommended for use with plant cell extracts. Cocktail contains six protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of aspartic, cysteine, serine, and metalloproteases as well as aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table on the right. One ml is recommended for about 30 g of various plant tissues.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	200 mM	Serine Proteases
Bestatin	308.4	10 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	3 mM	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	2 mM	Cysteine Proteases and Trypsin-like Proteases
<i>o</i> -Phenanthroline	198.2	500 mM	Metalloproteases
Pepstatin A	685.9	2 mM	Aspartic Proteases

### Protease Inhibitor Cocktail Set VII

Cat No. 539138

Form: Liquid

This cocktail is recommended for purification of proteins containing His•Tag® sequences. Cocktail contains five protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of cysteine, serine, aspartic, and thermolysin-like proteases and aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table on the right. One ml is sufficient for about 10 g cells.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
Bestatin	308.4	5 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Proteases
Pepstatin A	685.9	2 mM	Aspartic Proteases
Phosphoramidon, Disodium Salt	587.5	200 μM	Metalloendopeptidases

### Protease Inhibitor Cocktail Set VIII

Cat No. 539129

Form: Liquid

A DMSO solution of three protease inhibitors with selective specificity for the inhibition of cysteine proteases, including calpains, cathepsins, and papain. Each vial contains the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor	M.W.	Concentration in the Vial	Target Proteases
ALLN	383.5	1.56 mM	Calpain I/II, Cathepsin B, Cathepsin L, Cysteine Proteases
Cathepsin Inhibitor I	475.5	500 μM	Cathepsin B, Cathepsin L, Cathepsin S, Papain
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Protease

### Serine Protease Inhibitor Cocktail Set I

Cat No. 565000

Form: Lyophilized

A cocktail of four protease inhibitors that is useful for the inhibition of a broad range of serine proteases. Reconstitute each vial with 1 ml H<sub>2</sub>O to obtain a 100X stock solution. When diluted to 1X stock solution, the set will contain the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor	M.W.	1X stock concentration	Target Proteases
AEBSF, HCl	239.5	500 μM	Serine Proteases
Aprotinin, Bovine Lung	6512	420 nM	Serine Proteases and Esterases
Elastatinal	512.6	20 μM	An inhibitor of elastase-like serine protease.
GGACK	392.8	1 μM	An irreversible inhibitor of Urokinase and Factor Xa.

## Protease Inhibitor Set

Cat No. 539128

Form: Solid


This unique set, consisting of seven different inhibitors, allows you to perform easy, economical screening for a suitable inhibitor for your application.

Protease Inhibitor	M.W.	Quantity supplied	Target Proteases
AEBSF, HCl	239.5	50 mg	Serine Proteases
E-64 Protease Inhibitor	357.4	1 mg	Cysteine Proteases
EST	342.4	1 mg	Calpains
Leupeptin, Hemisulfate	475.6	5 mg	Cysteine Proteases and Trypsin-like Proteases
Pepstatin A	685.9	5 mg	Aspartic Proteases
TLCK, HCl	369.3	50 mg	Trypsin-like Serine Proteases
TPCK	351.5	250 mg	Chymotrypsin-like Proteases

## Protease Arrest™ Reagent

Cat No. 539124

An optimized concentration of various reversible and irreversible inhibitors to inhibit serine, cysteine, and calpain proteases. Suitable for the protection of proteins purified from animal tissues, plant tissues, yeast, and bacteria. Protease Arrest™ Reagent is provided as a 100X solution that when diluted in extraction buffer at pH 7.0–8.0 inhibits 95–98% of protease activity. EDTA is also provided separately to inhibit metalloproteases.



# Inhibitor Sourcebook™

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# Properties of Selected Protease Inhibitors

Protease Inhibitor	Cat. No.	Target Protease Class and Mechanism of Action	Solubility	Suggested Concentration	Size
AEBSF, Hydrochloride	101500	Water soluble, non-toxic alternative to PMSF. Irreversible inhibitor of serine proteases. Reacts covalently with a component of the active site. Inhibits chymotrypsin, kallikrein, plasmin, trypsin and related thrombolytic enzymes.	H <sub>2</sub> O	0.1–1 mM	50 mg 100 mg 500 mg 1 g
ALLN	208719	Inhibits plasmin, thrombin, calpain I (K <sub>i</sub> = 190 nM), calpain II (K <sub>i</sub> = 220 nM), cathepsin B (K <sub>i</sub> = 150 nM), and cathepsin L (K <sub>i</sub> = 500 pM).	Methanol, Ethanol, DMSO	0.2–2 μM	5 mg 25 mg
Aprotinin, Bovine Lung	616370	A serine protease inhibitor that acts as a competitive and reversible inhibitor of proteolytic and esterolytic activity. In cell cultures, extends the life of cells and prevents proteolytic damage to intact cells.	H <sub>2</sub> O	0.6–2.0 μg/ml	10 mg 20 mg 100 mg
Aprotinin, Recombinant	616371	A serine protease inhibitor that acts as a competitive and reversible inhibitor of proteolytic and esterolytic activity. In cell cultures, extends the life of cells and prevents proteolytic damage to intact cells.	H <sub>2</sub> O	0.6–2.0 μg/ml	1 mg 5 mg 25 mg
Bestatin	200484	Binds to cell surfaces and inhibits cell surface aminopeptidases, notably aminopeptidase B and leucine aminopeptidase. Activates macrophages and T lymphocytes. Has antitumor properties.	Methanol	1–10 μM	10 mg
Cathepsin Inhibitor I	219415	Selectively inhibits cathepsin B (k <sub>2</sub> /K <sub>i</sub> = 6.9 × 10 <sup>3</sup> M <sup>-1</sup> sec <sup>-1</sup> ), cathepsin L (k <sub>2</sub> /K <sub>i</sub> = 3.1 × 10 <sup>5</sup> M <sup>-1</sup> sec <sup>-1</sup> ), cathepsin S (k <sub>2</sub> /K <sub>i</sub> = 6.6 × 10 <sup>4</sup> M <sup>-1</sup> sec <sup>-1</sup> ), and papain (k <sub>2</sub> /K <sub>i</sub> = 1.8 × 10 <sup>3</sup> M <sup>-1</sup> sec <sup>-1</sup> ).	DMSO, Ethanol	100–200 μM	1 mg
E-64 Protease Inhibitor	324890	An irreversible cysteine protease inhibitor that has no action on cysteine residues in other proteins. Specific active site titrant.	H <sub>2</sub> O, DMSO	1–10 μM	1 mg 5 mg 25 mg
EDTA, Disodium Salt, Molecular Biology Grade	324503	A reversible metalloprotease inhibitor. A chelator that may interfere with other metal ion-dependent biological processes.	H <sub>2</sub> O	1–10 mM	100 g 1 Kg
Elastatinal	324691	A competitive inhibitor of elastase (K <sub>i</sub> = 240 nM).	H <sub>2</sub> O, DMSO, Ethanol	0.5–2 μg/ml	5 mg
EST	330005	A membrane-permeable calpain inhibitor.	Ethanol	20–50 μg/ml	1 mg
GGACK	347436	An irreversible inhibitor of Urokinase (IC <sub>50</sub> < 1 μM).	H <sub>2</sub> O	1–10 μM	5 mg
Leupeptin, Hemisulfate	108975	A reversible inhibitor of trypsin-like proteases and cysteine proteases, including endoproteinase Lys-C, papain, cathepsin B, trypsin, kallikrein, and thrombin.	H <sub>2</sub> O	10–100 μM	5 mg 10 mg 25 mg 50 mg 100 mg
Pepstatin A	516481	A reversible inhibitor of aspartic protease. Inhibits cathepsin D, cathepsin G, pepsin, and renin.	DMSO, Methanol	~ 1 μM	5 mg 25 mg 100 mg
o-Phenanthroline	516705	A metalloprotease inhibitor.	DMSO, Ethanol, H <sub>2</sub> O		500 mg
Phosphoramidon, Disodium Salt	525276	A highly specific inhibitor of thermolysin. Also inhibits the conversion of big endothelin-1 to endothelin-1.	H <sub>2</sub> O, DMSO, Methanol	1–10 μM	5 mg
TLCK, HCl	616382	An irreversible inhibitor of trypsin-like serine proteases. Inactivates trypsin, specifically and irreversibly. Does not have any significant inhibitory effect on chymotrypsin.	H <sub>2</sub> O	10–100 μM	50 mg 250 mg
TPCK	616387	An irreversible inhibitor of chymotrypsin. Useful for inhibiting chymotrypsin activity in trypsin preparations.	Ethanol, Methanol	10–100 μM	250 mg 1 g

# Protease Inhibitors | Frequently Asked Questions

## What criteria should I use when selecting a protease inhibitor?

When processing cells or tissues, you should assume that active proteases are present. Therefore, you should include protease inhibitors in the early steps of sample preparation. For best results, add protease inhibitors to the protein solution sample just before use to ensure the maximum activity for the cocktail during sample preparation. Typically, inhibitors should not be dissolved in buffers and stored for long periods of time. Since different cells and tissue types express different protease profiles, our cocktails are formulated with different sets of inhibitors. For example, serine proteases are widely distributed in all cells; bacterial cells contain higher levels of serine and metalloproteases; animal tissue extracts are rich in serine, cysteine, and metalloproteases, and plant extracts contain higher quantities of serine and cysteine proteases. Other selection criteria include what type of method is being used. EDTA is typically not desirable in 2-D gel electrophoresis studies.

## I currently use protease inhibitor cocktail tablets. Is it easy to switch to ready-to-use liquid cocktails?

Yes, in fact, there are several benefits to using pre-made liquid protease inhibitor cocktails. In general, liquid cocktails are:

- Stable - our cocktails offer a greater degree of stability compared to tablets dissolved in medium
- Efficient - the liquid cocktail format allows immediate exposure of inhibitors to proteases present in samples
- Flexible and economical - add only protocol-specific cocktail volumes as necessary
- Easy-to-use - working reagents are dissolved and ready to use
- Consistent - the same quantity of inhibitor is always dispensed



For additional information on our  
Protease and Phosphatase Inhibitor Cocktails  
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[www.emdbiosciences.com/inhibitorcocktails](http://www.emdbiosciences.com/inhibitorcocktails)



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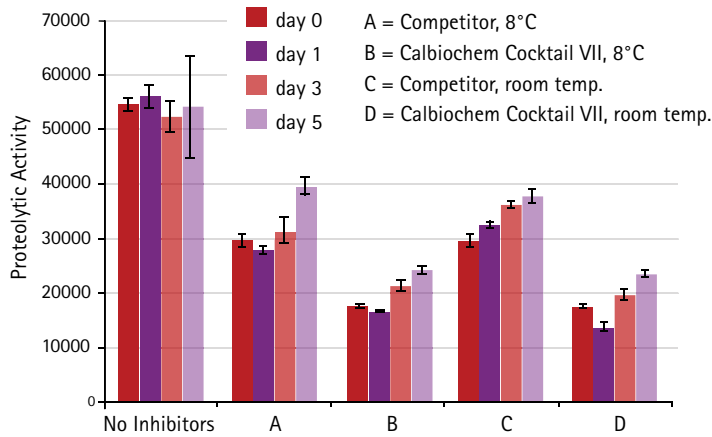




# Protease Inhibitors | Frequently Asked Questions

I work in an automation lab and want to use the cocktails on a liquid handling workstation. Are Calbiochem® protease inhibitor cocktails stable?

Our cocktails are compatible for use on liquid handlers and are stable, as shown in the data below.



## Stability of Protease Inhibitor Dilutions in BugBuster®

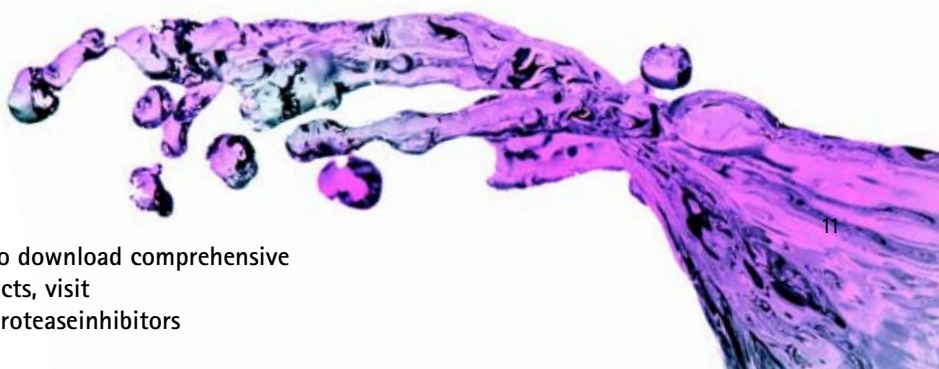
Protease inhibitors were diluted to the prescribed working concentration (Competitor; Calbiochem® Cocktail VII, Cat. No. 539138). The ability of the inhibitors to inhibit the proteolytic activity of PRONASE® (Cat. No. 537088) was measured by using the Universal HT Protease Assay on days zero, one (24 h post dilution), three (72 h) and five (120 h). The Universal HT Protease Assay quantifies protease activity using a fluorescein thiocarbonyl-casein derivative (FTC-casein). Proteolytic activity liberates FTC-labeled peptides, which results in enhanced fluorescence (*Ex.max: 495 nm; Em.max: 525 nm*). Addition of the protease inhibitor cocktails inhibits the proteolytic activity of the PRONASE® (Cat. No. 537088), resulting in reduced fluorescence. On day 1, for samples incubated at 8°C, the competitor tablet inhibited the proteolytic activity by 50% and the Calbiochem® Cocktail VII inhibited the proteolytic activity by 70%. On day 5, for samples incubated at 8°C, the competitor tablet caused a 29% decrease in proteolytic activity in comparison to the Calbiochem® cocktail VII, which caused a 57% decrease in proteolytic activity. The data shows that the efficiency of the cocktail remained higher than the competitor's tablet in this study.

# Phosphatase Inhibitor Cocktails

Phosphorylation and dephosphorylation of proteins mediate signal transduction events that control a multitude of cellular processes such as cell division, cell proliferation, apoptosis, etc. Target proteins are phosphorylated by protein kinases that transfer a phosphate group to a specific protein, typically at serine, threonine, or tyrosine residues. The covalent attachment of a phosphoryl group changes the conformation of the protein and alters its ability to interact with a ligand. The phosphate group is removed by the action of protein phosphatases, that restore the protein to its original dephosphorylated state. During the extraction of phosphorylated proteins from cell and tissue lysates, it is highly critical that the phosphorylation state of the proteins be preserved. We offer five different Phosphatase Inhibitor cocktails and a PhosphoSafe™ Extraction Reagent that help protect phosphoproteins from different families of phosphatases as shown in the selection guide below.

## Selection Guide for the Use of Specialized Phosphatase Inhibitor Cocktails

Product	Cat. No.	Recommended Application
Phosphatase Inhibitor Cocktail Set I	524624	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A <b>Not available for sale outside of the United States</b>
Phosphatase Inhibitor Cocktail Set II	524625	Protection against acid and alkaline phosphatases and Protein Tyrosine Phosphatases (PTPs)
Phosphatase Inhibitor Cocktail Set III	524627	Protection against acid, alkaline and Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)
Phosphatase Inhibitor Cocktail Set IV	524628	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A
Phosphatase Inhibitor Cocktail Set V	524629	Protection against acid and alkaline phosphatases, and Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)
PhosphoSafe™ Extraction Reagent	71296	Protection against Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)



For our current pricing or to download comprehensive information on these products, visit [www.emdbiosciences.com/proteaseinhibitors](http://www.emdbiosciences.com/proteaseinhibitors)



### Phosphatase Inhibitor Cocktail Set I

Cat No. 524624

Form: Liquid

A cocktail of three inhibitors that will inhibit alkaline phosphatases as well as serine/threonine protein phosphatases such as PP1 and PP2A. Each vial contains 1 ml of phosphatase inhibitor cocktail reconstituted in DMSO. The concentrations of the individual inhibitors are shown in the table on the right. Note: 1 set = 5 x 1 ml.

**Not available for sale outside of the United States.**

Phosphatase Inhibitor	M.W.	Concentration in the Vial	Target Phosphatases
(-)- <i>p</i> -Bromotetramisole oxalate	373.2	2.5 mM	Alkaline phosphatases
Cantharidin	196.2	500 μM	PP1 and PP2A
Microcystin LR, <i>Microcystis aeruginosa</i>	995.2	500 nM	PP1 and PP2A

### Phosphatase Inhibitor Cocktail Set II

Cat No. 524625

Form: Liquid

A cocktail of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases (PTPs). Suitable for use with tissue and cell extracts, including extracts containing detergents. Each vial contains 1 ml aqueous solution of the phosphatase inhibitor cocktail. The concentrations of the individual inhibitors are shown in the table on the right. Note: 1 set = 5 x 1 ml.

Phosphatase Inhibitor	M.W.	Concentration in the Vial	Target Phosphatases
Imidazole	68.1	200 mM	Alkaline phosphatase
Sodium Fluoride	42.0	100 mM	Acid phosphatase
Sodium Molybdate	205.9	115 mM	Acid phosphatase
Sodium Orthovanadate	183.9	100 mM	Protein tyrosine phosphatase, Alkaline Phosphatase
Sodium Tartrate, Dihydrate	230.1	400 mM	Acid phosphatase

### Phosphatase Inhibitor Cocktail Set III

Cat No. 524627

Form: Liquid

A cocktail of four phosphatase inhibitors for broad-spectrum inhibition of both serine/threonine and protein tyrosine phosphatases. Available as a 1 ml vial or as a set of five 1 ml vials. Each vial contains 1 ml aqueous solution of the phosphatase inhibitor cocktail. The concentrations of the individual inhibitors are shown in the table on the right.

Note: 1 set = 5 x 1ml.

Phosphatase Inhibitor	M.W.	Concentration in the Vial	Target Phosphatases
Sodium Fluoride	42.0	50 mM	Acid phosphatases
Sodium Orthovanadate	183.9	1 mM	Protein tyrosine phosphatase, Alkaline phosphatase
Sodium Pyrophosphate, decahydrate	416.1	10 mM	PP1 and PP2A
β-Glycerophosphate	306.1	10 mM	Ser/Thr phosphatases

### Phosphatase Inhibitor Cocktail Set IV

Cat No. 524628

Form: Liquid

A cocktail of three phosphatase inhibitors for the inhibition of both serine/threonine and alkaline phosphatases.

Available as a 1 ml vial or as a set of five 1 ml vials. Each vial contains a 1 ml solution phosphatase inhibitor cocktail.

The concentrations of the individual inhibitors are shown in the table on the right. Note: 1 set = 5 x 1ml.

Phosphatase Inhibitor	M.W.	Concentration in the Vial	Target Phosphatases
(-)- <i>p</i> -Bromotetramisole oxalate	373.2	2.5 mM	Alkaline phosphatases
Cantharidin	196.2	500 $\mu$ M	PP1 and PP2A
Calyculin A, <i>Discodermia calyx</i>	1009.2	1 $\mu$ M	PP1 and PP2A

### Phosphatase Inhibitor Cocktail Set V

Cat No. 524629

Form: Liquid

A cocktail of four phosphatase inhibitors for the inhibition of both serine/threonine and protein tyrosine phosphatases.

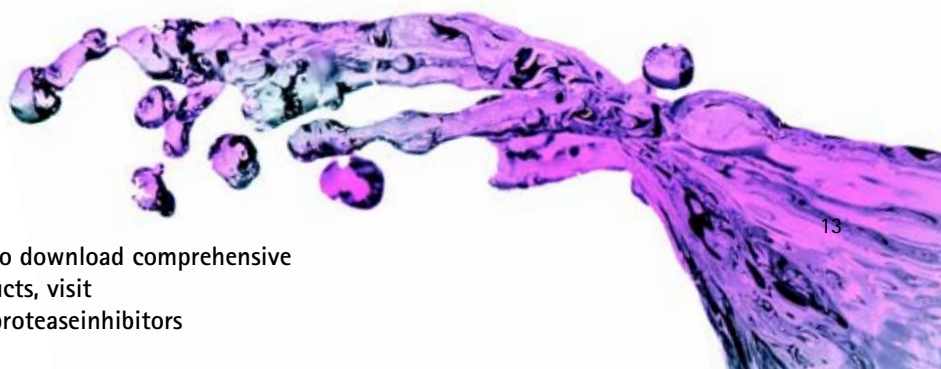
Available as 1 ml or as a set of five 1 ml vials. Each vial contains a 1 ml solution phosphatase inhibitor cocktail. The concentrations of the individual inhibitors are shown in the table on the right. Note: 1 set = 5 x 1ml.

Phosphatase Inhibitor	M.W.	Concentration in the Vial	Target Phosphatases
Sodium Fluoride	41.99	250 mM	Acid phosphatases
Sodium Orthovanadate	183.9	50 mM	Protein Tyrosine Phosphatase, Alkaline Phosphatase
Sodium Pyrophosphate, Decahydrate	446.06	50 mM	Ser/Thr Phosphatases
$\beta$ -Glycerophosphate	306.1	50 mM	Ser/Thr Phosphatases

### PhosphoSafe™ Extraction Reagent

Cat No. 71296

PhosphoSafe™ Extraction Reagent efficiently extracts cytosolic proteins from mammalian and insect cells while preserving their phosphorylation state. This reagent contains the same formulation as CytoBuster™ Protein Extraction Reagent, but also includes four phosphatase inhibitors: sodium fluoride, sodium vanadate,  $\beta$ -glycerophosphate, and sodium pyrophosphate. PhosphoSafe™ Extraction Reagent is compatible with kinase assays, protein interaction analysis, and other applications.



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## Selected Protein Phosphatase Inhibitors

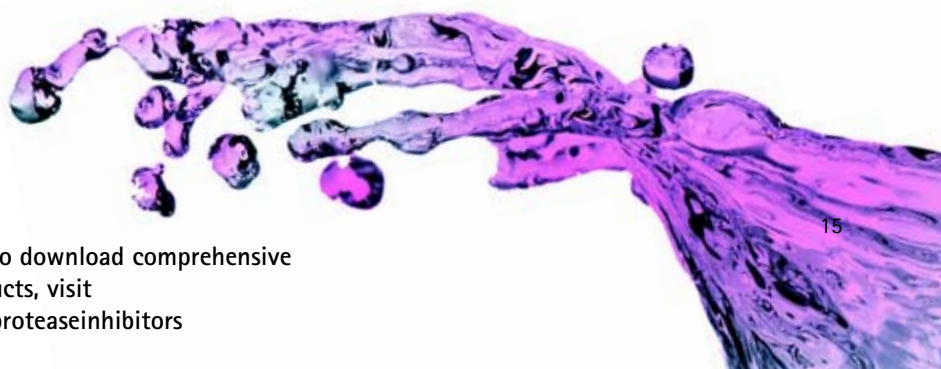
### Selected Protein Tyrosine Phosphatase Inhibitors

Product	Cat. No.	Size	Comments
3,4-Dephostatin	263202	1 mg	A PTPase inhibitor ( $IC_{50} = 18 \mu M$ ) that enhances growth factor-induced differentiation in PC12 cells. Exhibits greater stability than Dephostatin (Cat. No. 263200)
3,4-Dephostatin, Ethyl-	263203	1 mg	A potent inhibitor of PTP1B ( $IC_{50} = 3.2 \mu M$ ). A more stable ethyl analog of 3,4-Dephostatin (Cat. No. 263202).
bpV(bipy)	203694	5 mg	Blocks insulin receptor dephosphorylation ( $K_i = 100 nM$ ) and inhibits PTEN ( $IC_{50} = 18 nM$ )
bpV(phen)	203695	10 mg	A potent inhibitor of PTEN ( $IC_{50} = 38 nM$ )
bpV(pic)	203705	5 mg	A potent inhibitor of PTEN ( $IC_{50} = 31 nM$ )
Dephostatin	263200	1 mg	A competitive PTP inhibitor ( $IC_{50} = 7.7 \mu M$ )
mpV(pic)	475950	10 mg	A PTPase inhibitor and an activator of insulin receptor kinase
Phenylarsine Oxide	521000	250 mg	A PTP inhibitor ( $IC_{50} = 18 \mu M$ ) that blocks TNF- $\alpha$ -dependent activation of NF- $\kappa B$ in human myeloid ML-1a cells
Phosphotyrosine Phosphatase Inhibitor Set (Vanadium)	525325	1 set	A broad-spectrum potent inhibitor of PTPases, a potent inhibitor of PTEN ( $IC_{50} = 38 nM$ ), and an activator of insulin receptor kinase
PRL-3 Inhibitor	539808	10 mg	A cell-permeable and potent inhibitor of hPRL-3 ( $IC_{50} = 900 nM$ ),
PTP1B Inhibitor	539741	5 mg	A selective, reversible and non-competitive allosteric inhibitor of PTP1B ( $IC_{50} = 4 \mu M$ and $8 \mu M$ for PTP1B <sub>403</sub> and PTP1B <sub>298</sub> , respectively)
PTP CD45 Inhibitor	540215	1 mg	A cell-permeable, potent, selective, competitive, and reversible inhibitor of PTP1B ( $IC_{50} = 200 nM$ using pNPP as substrate)
PTP Inhibitor I	540200	10 mg	A cell-permeable, potent, and covalent protein tyrosine phosphatase (PTP) inhibitor. The inhibition can be reversed by irradiation of the inactivated PTP at 350 nm
PTP Inhibitor II	540205	25 mg	A cell-permeable, potent, and covalent protein tyrosine phosphatase (PTP) inhibitor. The inhibition can be reversed by irradiation of the inactivated PTP at 350 nm.
PTP Inhibitor III	540210	10 mg	A cell-permeable, potent, and covalent protein tyrosine phosphatase (PTP) inhibitor. The inhibition can be reversed by irradiation of the inactivated PTP at 350 nm.
PTP Inhibitor IV	540211	10 mg	A potent, reversible, competitive, and active-site directed inhibitor of PTPases ( $IC_{50} = 1.8, 2.5, 8.4, 13, 20, 6.4,$ and $6.7 \mu M$ for SHP-2, PTP1B, PTP- $\epsilon$ , PTP-Meg-2, PTP- $\sigma$ , PTP- $\beta$ , and PTP- $\mu$ , respectively).
RK-682, <i>Streptomyces</i> sp.	557322	200 $\mu g$	A limited cell-permeable, specific and noncompetitive inhibitor of PTPases. Inhibits dephosphorylation activity of CD45 ( $IC_{50} = 54 \mu M$ ) and VHR (vaccinia H1-related; $IC_{50} = 2 \mu M$ )
Sodium Orthovanadate	567540	5 gm	A broad-spectrum potent inhibitor of PTPases
Sodium Stibogluconate	567565	1 gm	A reversible PTP inhibitor that blocks SHP-1 activity (99% inhibition at $\sim 11 \mu M$ ). At higher concentrations ( $\sim 110 \mu M$ ) also inhibits SHP-2 and PTP1B
Suramin, Sodium Salt	574625	50 mg 200 mg	A reversible and competitive inhibitor of PTPases

# Selected Protein Phosphatase Inhibitors

## Selected Serine/Threonine Phosphatase Inhibitors

Product	Cat. No.	Size	Comments
Calcineurin Autoinhibitory Peptide	207000	250 µg	A potent inhibitor to the C-terminal domain (residues 457-482) of the calmodulin-binding domain of calcineurin (PP2B)
Calcineurin Autoinhibitory Peptide, Cell-permeable	207001	1 mg	A cell-permeable inhibitor to the C-terminal domain (residues 457-482) of the calmodulin-binding domain of calcineurin (PP2B)
Calyculin A, <i>Discodermia calyx</i>	208851	10 µg	A cell-permeable inhibitor of PP2A (IC <sub>50</sub> = 0.5 - 1.0 nM) and PP1 (IC <sub>50</sub> = 2 nM)
Cantharidic Acid	210150	10 mg	A potent inhibitor of PP2A (IC <sub>50</sub> = 50 nM)
Cantharidin	210155	20 mg	A potent inhibitor of PP2A (IC <sub>50</sub> = 40 nM) and PP1 at higher concentrations (IC <sub>50</sub> = 473 nM).
Cypermethrin	239900	10 mg	A potent inhibitor of PP2B (IC <sub>50</sub> = 1 nM)
Deltamethrin	253300	10 mg	A potent inhibitor of PP2B (IC <sub>50</sub> = 100 pM)
Endothall	324760	20 mg	A specific inhibitor of PP2A (IC <sub>50</sub> = 90 nM)
Fenvalerate	341380	25 mg	A potent inhibitor of PP2B (IC <sub>50</sub> = 2-4 nM)
Fostriecin, Sodium Salt, <i>Streptomyces pulveraceus</i>	344280	10 µg	A potent PP2A inhibitor (IC <sub>50</sub> = 3.2 nM) and a PP1 inhibitor at much higher concentrations (IC <sub>50</sub> = 131 µM).
InSolution™ Microcystin-LR, <i>Microcystis aeruginosa</i>	475821		A potent inhibitor of PP1 (IC <sub>50</sub> = 1.7 nM) and PP2A (IC <sub>50</sub> = 40 pM). A 2.5 mM (250 µg/100 µl) solution. <b>Not available for sale outside of the United States</b>
InSolution™ Nodularin, <i>Nodularia spumigena</i>	488003	50 µg	A potent and non-covalent-binding inhibitor of PP2A and PP1 (IC <sub>50</sub> = 33 pM and 1.6 nM, respectively)
InSolution™ Okadaic Acid, <i>Prorocentrum</i> sp.	495609	25 µg	A potent inhibitor of PP1 (IC <sub>50</sub> = 10-15 nM) and PP2A (IC <sub>50</sub> = 100 pM). A 250 µM (25 µg/124 µl) solution.
Microcystin-LF, <i>Microcystis aeruginosa</i>	475814		A cell-permeable and potent inhibitor of PP1 (IC <sub>50</sub> = 1.7 nM) and PP2A (IC <sub>50</sub> = 40 pM). <b>Not available for sale outside of the United States</b>
Microcystin-LR, <i>Microcystis aeruginosa</i>	475815		A potent inhibitor of PP1 (IC <sub>50</sub> = 1.7 nM) and PP2A (IC <sub>50</sub> = 40 pM). <b>Not available for sale outside of the United States</b>
Microcystin-LW, <i>Microcystis aeruginosa</i>	475818		A cell-permeable and potent inhibitor of PP1 (IC <sub>50</sub> = 1.7 nM) and PP2A (IC <sub>50</sub> = 40 pM). <b>Not available for sale outside of the United States</b>
Microcystin-RR, <i>Microcystis aeruginosa</i>	475816		A potent inhibitor of PP2A (IC <sub>50</sub> = 3.4 nM) <b>Not available for sale outside of the United States</b>
NIPP-1, His•Tag®, Bovine Thymus, Recombinant, <i>E. coli</i>	482251	1 µg	A potent and specific inhibitor of PP1 (K <sub>i</sub> = 1-10 pM). Can be used to distinguish the effect of PP1 from PP2A, PP2B, and PP2C
Okadaic Acid, Ammonium Salt	459616	25 µg	A water-soluble inhibitor of PP1 and PP2A
Okadaic Acid, Potassium Salt	459618	50 µg	A water-soluble inhibitor of PP1 and PP2A
Okadaic Acid, <i>Prorocentrum</i> sp.	495604	10 µg 25 µg 100 µg	A potent inhibitor of PP1 (IC <sub>50</sub> = 10-15 nM) and PP2A (IC <sub>50</sub> = 100 pM)
Okadaic Acid, Sodium Salt	459620	25 µg	A water-soluble inhibitor of PP1 and PP2A
PP2A Inhibitor I, GST-Fusion, Human Kidney, Recombinant, <i>E. coli</i>	539552	1 µg	A potent inhibitor of all forms of PP2A (IC <sub>50</sub> = ~100 pM)
PP2A Inhibitor I, GST-Fusion, Human, Recombinant, <i>E. coli</i>	539620	250 ng	A potent inhibitor of all forms of PP2A (IC <sub>50</sub> = ~100 pM)
PP12, Human, Recombinant, <i>E. coli</i>	539638	100 µg	A regulatory subunit of the cytosolic type 1 PP1 that inhibits the activity of the free catalytic subunit of PP1 (IC <sub>50</sub> = 1 nM)
PP12, Rabbit Muscle, Recombinant, <i>E. coli</i>	539516	20 µg	A specific inhibitor of PP1 (IC <sub>50</sub> = 2 nM).
Tautomycin, <i>Streptomyces spiroverticillatus</i>	580551	50 µg	Inhibits PP1 (IC <sub>50</sub> = 1 nM), PP2A (IC <sub>50</sub> = 10 nM), and smooth muscle endogenous phosphatase (IC <sub>50</sub> = 6 nM)



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