

# Protein Phosphatases in Signal Transduction

Phosphorylation and dephosphorylation of proteins mediate signal transduction events that control a multitude of cellular processes.<sup>1,2</sup> Target proteins are phosphorylated at specific serine, threonine, or tyrosine sites by protein kinases and the phosphate group is removed by the action of specific protein phosphatases. The activities of the cognate protein kinase or phosphatase acting on a particular site are well-regulated.<sup>3</sup> Protein phosphatases (PP) are classified based on their substrate specificity, dependence on metal ions, and sensitivity to inhibitory agents. The activities of PP1 and PP2A are independent of metal ions.<sup>4,5</sup> The catalytic subunit of PP1 is bound to the regulatory subunits that determine the subcellular localization and activity of the enzyme.<sup>6</sup> PP2A is inactivated by transient phosphorylation of tyrosine residues on the molecule.<sup>7</sup> PP2B, also known as calcineurin, consists of a catalytic subunit (A-subunit, 61 kDa) and a regulatory

subunit (B-subunit, 19 kDa). It is dependent on the Ca<sup>2+</sup>-calmodulin complex for complete activation.<sup>3</sup>

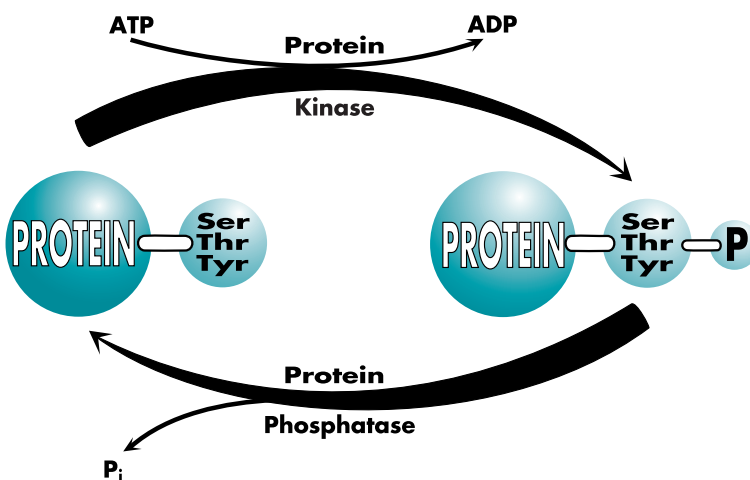
Over 40 protein tyrosine phosphatases (PTPs) have been characterized thus far. They possess a 230-amino acid catalytic domain and contain a number of regulatory subunits that appear to be essential for subcellular localization and regulation of enzymatic activity.<sup>4,8</sup> The table below outlines the salient features of the various protein phosphatases involved in signal transduction.

The termination of tyrosine phosphorylation on proteins is achieved by protein tyrosine phosphatases. Another class of phosphatases exhibits dual specificities (phosphoserine/phosphothreonine and phosphotyrosine). They are largely involved in the activation of cyclin-dependent kinases<sup>9</sup> and activation of MAP kinases.<sup>10</sup> Deregulation of protein phosphatases has been linked to various disease

states including cancer and diabetes. Recently, PTP 1B was shown to be a negative regulator of the insulin signaling pathway<sup>11,12</sup>, suggesting that PTP 1B inhibitors (such as DMHV) may be beneficial in the treatment of type 2 diabetes.

## Cited References:

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## Various Protein Phosphatases Involved in Signal Transduction

Protein Phosphatase	Localizations	Regulatory Subunits	Potent Inhibitors
PP1	Cytosol, nucleus, myofibrils, glycogen particles	G and M targeting subunits	Calyculin A, Nodularin, NIPP-1
PP2A	Cytosol, mitochondria, nucleus	A and B subunits	Calyculin A, Microcystins, Nodularin, Okadaic Acid, Endothall
PP2B	Cytosol, plasma membrane, synaptosome, nucleus	B subunit; calmodulin	Cyclosporin A and FK 506/Immunophilin complexes, Cypermethrin, Deltamethrin, Fenvalerate
PTP	Plasma membrane, nucleus	various	bpV(phen), Dephostatatin, mpV(pic) DMHV, Sodium Orthovanadate

## Phosphatases

Phosphatase	Cat. No.	Comments	Size
Phosphatase, Acid, Potato	<b>524528</b>	May be used for the removal of phosphate groups from phosphoproteins, such as $\beta$ -casein, ovalbumin, and pepsinogen. <b>Activity: <math>\geq 6</math> units/mg.</b>	50 U
Phosphatase, Alkaline, Calf Intestine	<b>524572</b>	Homodimeric enzyme that hydrolyzes phosphate esters of primary and secondary alcohols, amines, and phenols. Useful in hydrolysis of 5'-terminal phosphates of DNA and RNA. Active site contains a reactive serine. Has an optimal pH of 8.0 - 10.5 and a pI of 5.7. M.W. 140 kDa. <b>Activity: <math>\geq 1500</math> units/mg.</b>	1 KU 5 KU
Phosphatase, Alkaline, Calf Intestine, Molecular Biology Grade	<b>524576</b>	Also suitable for use in reactions containing nucleotide sugars. Has an optimal pH of 8.0 - 10.5. M.W. 140 kDa. <b>Activity: <math>\geq 2000</math> units/mg.</b>	1 KU
Phosphatase, Alkaline, <i>E. coli</i>	<b>524545</b>	A dimeric zinc and magnesium-containing protein. A non-specific phospho-monoesterase. Has a pI of 4.5. M.W. 80 kDa. <b>Activity: <math>\geq 500</math> units/ml.</b>	50 U
Phosphatase, Alkaline, Human Placenta	<b>524604</b>	Homodimeric enzyme that hydrolyzes phosphate esters of primary and secondary alcohols, amines, and phenols. Useful in the hydrolysis of 5'-terminal phosphates of DNA and RNA. Active site contains a reactive serine. <b>Activity: <math>\geq 100</math> units/mg.</b>	100 U
Protein Phosphatase 1, $\alpha$ -isoform, Rabbit Muscle, Recombinant, <i>E. coli</i>	<b>539493</b>	Mn <sup>2+</sup> -dependent phosphatase that hydrolyzes phosphates on Ser and Thr residues. Assists in tobacco mosaic virus-mediated apoptosis. M.W. 37.5 kDa. <b>Activity: <math>\geq 500</math> units/ml.</b>	100 U 200 U
Protein Phosphatase 1, Catalytic Subunit, $\gamma$ -Isoform, Human, Recombinant, <i>E. coli</i>	<b>539555</b>	Major Ser/Thr phosphatase found in eukaryotes. Identical to the rat enzyme. M.W. 37 kDa. <b>Activity: <math>\geq 2</math> units/mg.</b>	10 $\mu$ g
Protein Phosphatase 1, Catalytic Subunit, His-Tag®, $\gamma 1$ -Isoform, <i>Xenopus</i> , Recombinant	<b>539527</b>	Virtually identical to mammalian PP1. Accounts for 40 – 60% of the phosphorylase and phosphatase activity in muscle and liver. M.W. 42 kDa. <b>Activity: <math>\geq 1000</math> units/mg.</b>	10 U
Protein Phosphatase 2A <sub>1</sub> , Bovine Kidney	<b>539508</b>	Ser/Thr phosphatase involved in numerous cellular processes. Has a catalytic subunit (36 kDa), a regulatory subunit (60 kDa), and a third subunit (55 kDa). M.W. 150 kDa. <b>Activity: <math>\geq 750</math> units/mg.</b>	1 $\mu$ g
Protein Phosphatase 2A <sub>2</sub> , Bovine Kidney	<b>539510</b>	Ser/Thr phosphatase involved in numerous cellular processes. Has a catalytic subunit (36 kDa) and a regulatory subunit (60 kDa). M.W. 96 kDa. <b>Activity: <math>\geq 750</math> units/mg.</b>	1 $\mu$ g
Protein Phosphatase 2B (Calcineurin), Human, Recombinant, <i>E. coli</i>	<b>539568</b>	Major Ca <sup>2+</sup> -calmodulin dependent Ser/Thr phosphatase with broad substrate specificity. Consists of a 60 kDa catalytic subunit and a 15 kDa regulatory subunit. Acts as a target of immunophilin/immunosuppressant complexes in T cells. M.W. 75 kDa. <b>Activity: <math>\geq 300,000</math> units/mg.</b>	5000 U
Protein Phosphatase, Lambda, Recombinant, <i>E. coli</i>	<b>539514</b>	Mn <sup>2+</sup> -dependent phosphatase that hydrolyzes phosphates on Ser, Thr, Tyr, or His residues. M.W. 25 kDa. <b>Activity: <math>\geq 300,000</math> units/mg.</b>	20 KU
Protein Tyrosine Phosphatase 1B, Human, Recombinant, <i>E. coli</i>	<b>539735</b>	A ubiquitous non-transmembrane PTP, useful for the study of tyrosine phosphatase kinetics. Suitable for screening inhibitors or determining substrate specificity. M.W. 37.4 kDa. <b>Activity: <math>\geq 50</math> units/mg.</b>	50 $\mu$ g
Protein Tyrosine Phosphatase, CD45, Human, Recombinant	<b>217614</b>	Corresponds to the cytoplasmic domain (amino acid residues 584 – 1281) of human CD45. Useful for regulation and inhibition studies. M.W. 95 kDa. <b>Activity: <math>\geq 20,000</math> units/mg.</b>	20 $\mu$ g
Protein Tyrosine Phosphatase, LAR, Human, Recombinant, <i>E. coli</i>	<b>539731</b>	Soluble catalytic domain (350 amino acid residues) of the human transmembrane leukocyte antigen-related tyrosine phosphatase. Involved in the modulation of insulin receptor signaling in intact cells. M.W. 40 kDa. <b>Activity: <math>\geq 5000</math> units/mg.</b>	200 U
Protein Tyrosine Phosphatase, T-Cell, Human, Recombinant, <i>E. coli</i>	<b>539732</b>	Truncated form of the human T cell phosphatase with an 11 kDa deletion from the C-terminus of the native protein. M.W. 38 kDa. <b>Activity: <math>\geq 15,000</math> units/mg.</b>	200 U
Protein Tyrosine Phosphatase, <i>Yersinia enterocolitica</i> , Recombinant, <i>E. coli</i>	<b>539734</b>	Catalytic domain of the <i>Yersinia</i> tyrosine phosphatase containing the C235R mutation. Suitable for dephosphorylation of phosphotyrosine residues in proteins. M.W. 51 kDa. <b>Activity: <math>\geq 50,000</math> units/mg.</b>	2000 U
Stp1, Low Molecular Weight Phosphatase, <i>Schizosaccharomyces pombe</i> , Recombinant	<b>570300</b>	Dual activity phosphatase that can remove both aryl phosphates (such as phosphotyrosine) and alkyl phosphates (such as phosphoserine) from proteins or peptide substrates. M.W. 17.4 kDa. <b>Activity: <math>\geq 6</math> units/mg.</b>	50 $\mu$ g

## Protein Phosphatase Inhibitors

Product	Cat. No.	M.W.	Comments	Size
bpV(bipy)	<b>203694</b>	380.3	A potent phosphotyrosine phosphatase inhibitor and insulin receptor kinase (IRK) activator.	5 mg
bpV(HOPic)	<b>203701</b>	419.3	A potent phosphotyrosine phosphatase inhibitor and insulin receptor kinase (IRK) activator.	5 mg
bpV(phen)	<b>203695</b>	404.3	Potent protein tyrosine phosphatase (PTP) inhibitor. Exhibits 1000-fold potency over sodium orthovanadate.	10 mg
Calcineurin Autoinhibitory Peptide	<b>207000</b>	2930.4	Specific calcineurin inhibitor. Inhibits Mn <sup>2+</sup> -stimulated PP2B activity (IC <sub>50</sub> = 10 μM using <sup>32</sup> P-myosin light chain as substrate) without affecting Ni <sup>2+</sup> -stimulated activity.	250 μg
Calyculin A	<b>208851</b>	1009.2	Cell-permeable. Phosphorylated polyketide. PP2A ~ PP1 >> PP2B (IC <sub>50</sub> for PP2A = 0.5 – 1.0 nM and for PP1 = 2.0 nM).	10 μg
Cantharidic Acid	<b>210150</b>	214.2	Terpenoid. Has high selectivity for PP2A (IC <sub>50</sub> = 50 nM).	10 μg
Cantharidin	<b>210155</b>	196.2	Cell-permeable. Terpenoid. PP2A > PP1 >> PP2B (IC <sub>50</sub> for PP2A = 40 nM and for PP1 = 473 nM).	20 mg
Cyclosporin A	<b>239835</b>	1202.6	Binds to cyclophilin in cell; the complex inhibits PP2B with nanomolar affinity.	100 mg
Cypermethrin	<b>239900</b>	416.3	Potent inhibitor of PP2B (IC <sub>50</sub> = 40 pM).	10 mg
Deltamethrin	<b>253300</b>	505.2	Potent inhibitor of PP2B (IC <sub>50</sub> = 100 pM).	10 mg, 50 mg
Dephostatin	<b>263200</b>	168.2	Protein tyrosine phosphatase (PTP) inhibitor (IC <sub>50</sub> = 7.7 μM).	1 mg
3,4-Dephostatin	<b>263202</b>	168.2	A protein tyrosine phosphatase inhibitor (IC <sub>50</sub> = 18 μM).	1 mg
1,4-Dimethylethanol	<b>311250</b>	214.2	A useful negative control for Cantharidic Acid (Cat. No. 210150), Cantharidin (Cat. No. 210155), and Endothall (Cat. No. 324760).	10 mg
DMHV	<b>322130</b>	390.2	A potent, cell-permeable, and reversible PTP inhibitor (IC <sub>50</sub> = 1 – 2 μM).	10 mg
Endothall	<b>324760</b>	186.2	A specific inhibitor of PP2A (IC <sub>50</sub> = 90 nM).	20 mg
Fenvalerate	<b>341380</b>	419.9	Potent inhibitor of PP2B (IC <sub>50</sub> = 2 – 4 nM).	25 mg
Fostriecin, Sodium Salt, <i>Streptomyces pulvaceus</i>	<b>344280</b>	452.4	A potent PP2A inhibitor (IC <sub>50</sub> = 3.2 nM). Inhibits PP1 only at higher concentrations (IC <sub>50</sub> = 131 μM).	10 μg
Microcystin-LA	<b>475813</b>	910.1	Potent inhibitor of PP1 (IC <sub>50</sub> = 2.0 nM) and PP2A (IC <sub>50</sub> = 40 pM). Does not enter some mammalian cells.	100 μg
Microcystin-LF	<b>475814</b>	986.2	A more cell-permeable analog of Microcystin-LR (Cat. No. 475815). Useful for studies in intact cells.	25 μg
Microcystin-LR	<b>475815</b>	995.2	Cyclic peptide. PP2A ~ PP1 >> PP2B (IC <sub>50</sub> for PP2A = 40 pM and for PP1 = 1.7 nM). Does not enter some mammalian cells.	500 μg
Microcystin-LW	<b>475818</b>	1025.2	A more cell-permeable analog of Microcystin-LR (Cat. No. 475815). Useful for studies in intact cells.	25 μg
Microcystin-RR	<b>475816</b>	1038.2	Cyclic peptide. PP2A ~ PP1 >> PP2B (IC <sub>50</sub> = 3.4 nM). Does not enter some mammalian cells.	250 μg
Microcystin-YR	<b>475819</b>	1045.2	Cyclic peptide. PP2A ~ PP1 >> PP2B. Does not enter some mammalian cells.	250 μg
mpV(pic)	<b>475950</b>	257.1	Potent PTP inhibitor. More potent for insulin receptor (IR) dephosphorylation than epidermal growth factor receptor (EGFR) dephosphorylation.	10 mg
α-Naphthyl Acid Phosphate, Monosodium Salt	<b>479775</b>	246.1	Broad-spectrum competitive protein phosphatase inhibitor.	5 g
NIPP-1, Bovine Thymus, Recombinant	<b>482250</b>	38,500	Potent, specific inhibitor of PP1 (K <sub>i</sub> = 1 - 10 pM). Suitable to distinguish PP1 activity from PP2A, PP2B, or PP2C activity.	1 μg
Nodularin	<b>488002</b>	825.0	Cyclic peptide. PP2A ~ PP1 >> PP2B. Does not enter some mammalian cells.	250 μg
1-Norokadaone	<b>490055</b>	759.0	Analog of Okadaic Acid (Cat. No. 495604) that lacks phosphatase activity. Suitable for a negative control.	50 μg
Okadaic Acid	<b>495604</b>	805.0	Cell-permeable. PP2A > PP1 >> PP2B (IC <sub>50</sub> for PP2A = 0.1 nM; for PP1 = 10 – 15 nM; and for PP2B = 5 μM).	10 μg, 100 μg
Okadaic Acid, Ammonium Salt	<b>459616</b>	822.0	Water-soluble form. Has greater stability in solution.	25 μg
Okadaic Acid, Potassium Salt	<b>459618</b>	843.1	Water-soluble form. Has somewhat greater stability in solution.	50 μg
Okadaic Acid, Sodium Salt	<b>459620</b>	827.0	Water-soluble form. Has greater stability in solution.	25 μg

## Protein Phosphatase Inhibitors (continued)

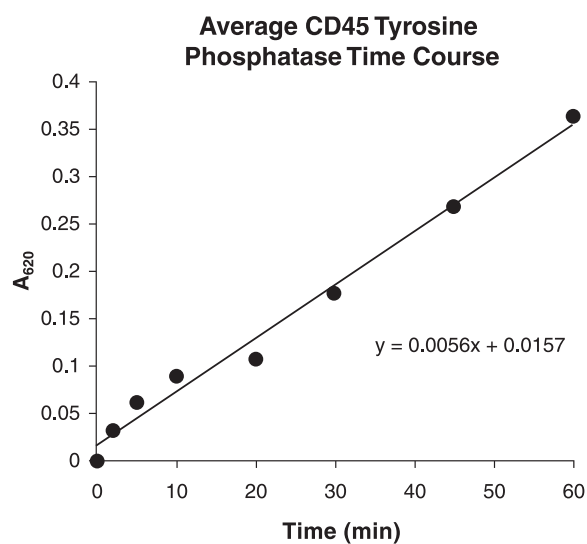
Product	Cat. No.	M.W.	Comments	Size
Okadaic Acid, 7,10,24,28-Tetraacetate	<b>495615</b>	973.2	Analog of okadaic acid (Cat. No. 495604) that lacks phosphatase activity. Suitable for a negative control.	50 µg
Phenylarsine Oxide	<b>521000</b>	168.0	Cell-permeable PTP inhibitor (IC <sub>50</sub> = 18 mM).	250 mg
5-Phosphatase Inhibitor	<b>524620</b>	432.4	Inhibits 5-phosphatase-catalyzed dephosphorylation of Ins(1,4,5)P <sub>3</sub> (K <sub>i</sub> = 4 µM).	1 mg
PP Inhibitor 2, Rabbit, Muscle Recombinant, <i>E. coli</i>	<b>539516</b>	22,800	Inhibits the catalytic subunit of PP1 (IC <sub>50</sub> = 2 nM).	20 µg
Protein Phosphatase 2A Inhibitor I <sub>1</sub> <sup>PP2A</sup> , Kidney	<b>539552</b>	30,000	A non-competitive inhibitor of PP2A (K <sub>i</sub> = 30 nM).	250 ng
Protein Phosphatase 2A Inhibitor I <sub>2</sub> <sup>PP2A</sup> , Human, Recombinant, <i>E. coli</i>	<b>539620</b>	39,000	A non-competitive inhibitor of PP2A.	250 ng
PTP Inhibitor I	<b>540200</b>	215.1	Potent, cell-permeable, and covalent PTP inhibitor. Binds SHP-1 (K <sub>i</sub> = 42 µM). Inhibition can be reversed by irradiation of the inactivated PTP at 350 nM.	10 mg
PTP Inhibitor II	<b>540205</b>	229.1	Potent, cell-permeable, and covalent PTP inhibitor. Lower affinity than PTP inhibitor I (K <sub>i</sub> = 128 µM) but has higher k <sub>inact</sub> (2.4 min <sup>-1</sup> vs. 0.4 min <sup>-1</sup> ). Inhibition can be reversed by irradiation of the inactivated PTP at 350 nM.	25 mg
PTP Inhibitor III	<b>540210</b>	273.1	Potent, cell permeable, and covalent PTP inhibitor. Lower affinity than PTP inhibitor I (K <sub>i</sub> = 193 µM) but has higher k <sub>inact</sub> (1.8 min <sup>-1</sup> vs. 0.4 min <sup>-1</sup> ). Inhibition can be reversed by irradiation of the inactivated PTP at 350 nM.	10 mg
Resmethrin	<b>554300</b>	338.4	Weak or inactive in calcineurin-related assays.	10 mg
RK-682	<b>557322</b>	368.5	A specific, non-cell-permeable inhibitor of protein tyrosine phosphatase. Inhibits dephosphorylation activity of CD45 (IC <sub>50</sub> = 54 µM) and VHR (vaccinia H1-related; IC <sub>50</sub> = 2.0 µM) <i>in vitro</i> .	200 µg
Sodium Orthovanadate	<b>567540</b>	183.9	Inhibitor of protein tyrosine phosphatases of general/broad specificity; potent inhibitor of alkaline phosphatase.	5 g

### Phosphatase Assay Kits

#### CD45 Tyrosine Phosphatase Assay Kit, Colorimetric

Colorimetric assay kit for measuring CD45 tyrosine phosphatase activity and inhibitor screening. The assay is performed in a 96-well microtiter plate format using pp60<sup>C-src</sup> C-terminal phosphopeptide as substrate. Kit also includes CD45 enzyme positive control, RWJ-60475 phosphatase inhibitor, phosphate standard, detection reagent, assay buffer, ½-volume microtiter plate, and a directional insert.

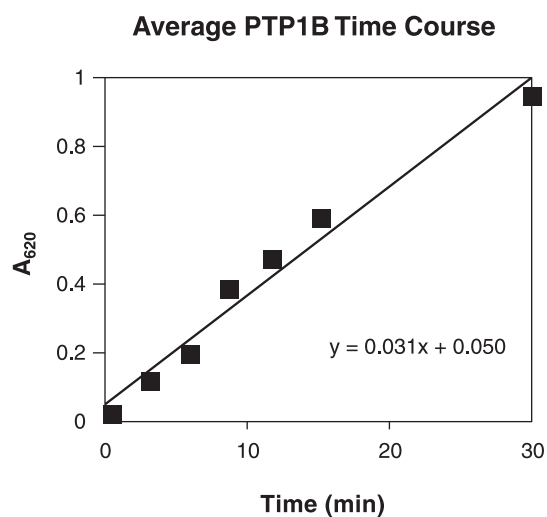
**Cat. No. 219454**



#### Protein Tyrosine Phosphatase 1B Assay Kit, Colorimetric

A colorimetric assay designed to measure PTP1B phosphatase activity of purified enzyme. This 96-well assay is useful for screening inhibitors and modulators of PTP1B activity. The kit includes human recombinant PTP1B enzyme (amino acid residues 1 - 322), the EGFR (988 - 998) phosphopeptide substrate (K<sub>m</sub> = 3.9 µM), the PTP1B inhibitor RK-682, phosphate standard, detection reagent, assay buffer, ½-volume microtiter plate, and a directional insert.

**Cat. No. 539736**



## Calcineurin Assay Kits

### Calcineurin Assay Kit, Colorimetric

A colorimetric assay designed to measure calcineurin activity. The assay is performed in a 96-well microtiter plate format using the RII phosphopeptide as substrate. The kit also includes human recombinant calcineurin (co-expressed calcineurin A $\alpha$  + N-myristoylated calcineurin B heterodimers), calmodulin, phosphate standard, detection reagent, assay buffer, 1/2-volume microtiter plate, and a directional insert.

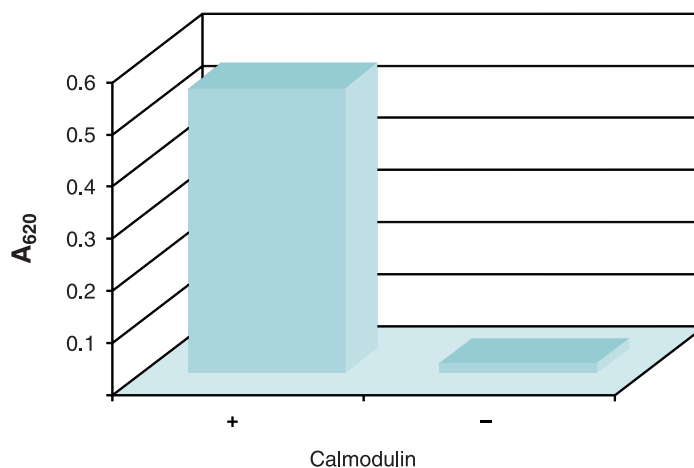
**Cat. No. 207005**

### Calcineurin Cellular Activity Assay Kit, Colorimetric

A complete colorimetric assay kit for measuring cellular calcineurin (PP-2B) phosphatase activity from cell or tissue extracts. It employs a convenient 96-well microtiter plate format with all reagents necessary for measuring calcineurin phosphatase activity, including human recombinant calcineurin, the RII phosphopeptide substrate, calmodulin, assay buffer, lysis buffer, EGTA buffer, protease inhibitor cocktail, okadaic acid, detection reagent, desalting column resin, chromatography column, 1/2-volume microtiter plate, and a directional insert.

**Cat. No. 207007**

### The Effect of Calmodulin on the Calcineurin Assay



## Antibodies and Blocking Peptides to Phosphatases

Product	Cat. No.	Size	Applications
Anti-NIPP-1, N-Terminal (341–351), Bovine Thymus (Rabbit)	482254	50 $\mu$ l	IB, IC, IFA, IP
NIPP-1 (341–351), Blocking Peptide, Bovine Thymus	482255	100 $\mu$ g	—
Anti-Protein Phosphatase 1 $\alpha$ , C-Terminal (Rabbit)	539517	100 $\mu$ l	IB, IP
Anti-Protein Phosphatase 1 $\beta$ , C-Terminal (Rabbit)	539537	100 $\mu$ l	IB, IP
Anti-Protein Phosphatase 1 $\gamma$ 1, C-Terminal (Rabbit)	539543	100 $\mu$ l	IB, IP
Anti-Protein Phosphatase 2A/A (7-19) (Rabbit)	539509	100 $\mu$ l	IB, IC
Protein Phosphatase 2A/A (7-19) Blocking Peptide	539519	100 $\mu$ g	—
Anti-Protein Phosphatase 2A/B $\alpha$ (14-27) (Rabbit)	539521	100 $\mu$ l	IB, IC
Protein Phosphatase 2A/B $\alpha$ (14-27) Blocking Peptide	539524	100 $\mu$ g	—
Anti-Protein Phosphatase 2A/B $\beta$ , N-Terminal (2–14) (Rabbit)	539545	100 $\mu$ l	IB
Anti-Protein Phosphatase 2A/B $\gamma$ , N-Terminal (53–66) (Rabbit)	539546	100 $\mu$ l	IB
Anti-Protein Phosphatase 2A/C (298–309) (Rabbit)	539525	100 $\mu$ l	IB, IC
Protein Phosphatase 2A/C (298–309) Blocking Peptide	539528	100 $\mu$ g	—
Anti-Protein Phosphatase 2A/C (17–87) (Ab-1) (Rabbit)	PC263	200 $\mu$ g	IB
Anti-Protein Phosphatase 2A/C (15–145) (Ab-1) (Rabbit)	PC264	200 $\mu$ g	IB
Anti-Protein Phosphatase 2B $\alpha$ (482–494) (Rabbit)	539530	100 $\mu$ l	IB, IC
Protein Phosphatase 2B $\alpha$ (482–494) Blocking Peptide	539531	100 $\mu$ g	—
Anti-Protein Phosphatase 2C $\alpha$ , $\beta$ , N-Terminal (23–35) (Rabbit)	539548	100 $\mu$ l	IB
Anti-Protein Phosphatase 2C $\gamma$ (Ab-1) (Mouse)	PH04	100 $\mu$ g	IB, IFA, IP
Anti-Protein Phosphatase X/C (294–307) (Rabbit)	539539	100 $\mu$ l	IB, IC
Protein Phosphatase X/C (294–307) Blocking Peptide	539540	100 $\mu$ g	—
Anti-Protein Phosphatase- $\mu$ (Ab-1) (Mouse)	PH05	100 $\mu$ g	IB, IP
Anti-Protein Tyrosine Phosphatase 1B (Ab-1) (Mouse)	PH01	100 $\mu$ g	IB, IFA, IP
Anti-Protein Tyrosine Phosphatase 1B (Ab-2) (Mouse)	PH02	100 $\mu$ g	IB, IFA, IP
Anti-T-Cell Protein Tyrosine Phosphatase (Ab-1) (Mouse)	PH03L	100 $\mu$ g	IB, IC, IP

IB = Immunoblotting; IC = Immunocytochemistry; IFA = Immunofluorescent Assay; IP = Immunoprecipitation



## Phosphatase Substrates

Substrate	Cat. No.	M.W.	Comments	Size
Calcineurin Substrate	<b>207008</b>	2192	Also called RII Phosphopeptide. An excellent substrate for PP2B. Phosphate release can be quantified colorimetrically using malachite green.	500 µg
4-Methylumbelliferyl-, phosphate, Free Acid	<b>474431</b>	256.2	Ultrasensitive substrate for fluorometric, phosphorometric, and spectrophotometric assays of phosphatases.	1 g
<i>p</i> -Nitrophenyl Phosphate	<b>487663</b>		Stable solution. Excellent substrate for alkaline-phosphatase-based ELISA assays. Produces soluble end product that can be read at ~405 nm.	10 ml
<i>p</i> -Nitrophenyl Phosphate, Disodium Salt	<b>4876</b>	263.1	Excellent substrate for alkaline-phosphatase-based ELISA assays. Produces soluble end product that can be read at ~405 nm.	1 g 5 g
<i>p</i> -Nitrophenyl Phosphate, DiTris Salt	<b>487655</b>	461.4	Excellent substrate for alkaline-phosphatase-based ELISA assays. Produces soluble end product that can be read at ~405 nm.	5 g 100 g
Protein Tyrosine Phosphatase Substrate	<b>539750</b>	1118	Sensitive substrate for the detection and characterization of a wide variety of intracellular and receptor-linked protein tyrosine phosphatases, particularly when limiting amounts of tissue extracts or immunoprecipitates are available.	500 µg
Protein Tyrosine Phosphatase Substrate II	<b>539738</b>	1863	PTP substrate containing multiple phosphorylated tyrosine residues. Derived from the insulin receptor β-subunit cytoplasmic domain, including Tyr <sup>1146</sup> , Tyr <sup>1150</sup> , and Tyr <sup>1151</sup> .	500 µg
Protein Tyrosine Phosphatase 1B Substrate	<b>539737</b>	1330	Excellent substrate for mammalian PTP 1B (Cat. No. 539735) and <i>Yersinia</i> PTP (Cat. No. 539734). Derived from an autophosphorylation site (Tyr <sup>992</sup> ) of EGFR.	1 mg
Protein Tyrosine Phosphatase 1B Substrate II	<b>539739</b>	1359	Fluorogenic substrate for PTP 1B ( $k_{cat}/K_m = 2.2 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ ). Hydrolysis is measured by the increase in tyrosine fluorescence which can be monitored at 305 nm following excitation at 280 nm.	1 mg
Protein Tyrosine Phosphatase Substrate III	<b>539740</b>	1441	Specific, sensitive fluorescence resonance energy transfer substrate for PTPs. The substrate sequence is similar to that surrounding the phosphotyrosine residue in Src.	500 µg
Raytide Substrate	<b>PK02</b>	2116	Modified gastrin analog that acts as a substrate for tyrosine kinases. Contains one acidic end for binding to phosphocellulose paper and one basic end. When labeled with <sup>32</sup> P-phosphate, provides an excellent phosphatase substrate for phosphorylation studies.	200 µg
Raytide EL Substrate	<b>PK04</b>	2476	Variation of Cat. No. PK02 with a higher labeling efficiency, when used as either a kinase or phosphorylase substrate.	200 µg
Raytide Control Substrate	<b>PK05</b>	2476	Negative control substrate for use with Cat. Nos. PK02 and PK04. Does not undergo phosphorylation.	100 µg

## Inhibitor Sets and Cocktails

### Protein Phosphatase Inhibitor Set

Convenient collection of several of our most popular inhibitors. Contains 10 mg cypermerthrin (Cat. No. 239900), 1 mg dephostatin (Cat. No. 263200); 10 µg okadaic acid (Cat. No. 495604), and 1 µg NIPP-1 (Cat. No. 482250).

**Cat. No. 539630**

**1 set**

### Phosphatase Inhibitor Cocktail I

Cocktail of three phosphatase inhibitors specially formulated for inhibition of alkaline phosphatases as well as serine/threonine protein phosphatases. Each vial contains 1 ml of phosphatase inhibitor cocktail solubilized in DMSO with the following components: 2.5 mM (-)-*p*-bromotetramisole oxalate (Cat. No. 203975), 500 µM cantharidin (Cat. No. 210155), and 500 nM microcystin-LR (Cat. No. 475815).

**Cat. No. 524624**

**5 x 1 ml**

### Phosphatase Inhibitor Cocktail II

Cocktail of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases. Each vial contains 1 ml aqueous solution of 200 mM imidazole (Cat. No. 4015), 100 mM sodium fluoride, 115 mM sodium molybdate, 100 mM sodium orthovanadate (Cat. No. 567540), and 400 mM sodium tartrate dihydrate.

**Cat. No. 524625**

**5 x 1 ml**

Please call our Technical Service Department or your local sales office for more information on these products.

<p><b>United Kingdom</b> Tel (0115) 943 0840 Fax (0115) 943 0951 customer.service@cnuuk.co.uk</p>	<p><b>USA, Canada, &amp; Mexico</b> Tel (800) 854-3417 Fax (800) 776-0999 technical@calbiochem.com</p>	<p><b>VWR Scientific Products</b> www.vwrsp.com or <b>VWR Canlab</b> www.vwrcanlab.com Tel (800) 932-5000</p>	<p><b>Germany</b> Tel (06196) 63955 Fax (06196) 62361 customer.service@calbiochem-novabiochem.de</p>
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