

## Continuous Fluorescent Intensity Phosphatase Assays

A Powerful Approach for Understanding Phosphatase and Phosphatase Inhibitor Activity

### Search Below to Match Your Kinase With Our Sensor Peptide Substrate

**Bulk Sensor Peptide (PN: *CSPS-AQTxxxxB*)** : Designed for higher throughput applications. 1mg is sufficient for ~2,000 assays in a low-volume 384-well plate (10µM sensor peptide in 25µL reaction volume). Available from 1mg to +200mg aliquots. We also offer optimized buffers and reagents (sold separately, refer to Table 6 below).

**Kits (PN: *CSPS-AQTxxxxK*)** : Designed for streamlined technology evaluation. Each kit enables 96 reactions in a low-volume 384-well plate (10µM sensor peptide in 25µL reaction volume). Includes all assay components (excluding your phosphatase). Refer to Table 5 below.

HGNC (Common) Name	HGNC Approved Full Name	Ser (S) Thr (T) Tyr (Y)	Sensor Peptide Product Number (Bulk, Kit)	Sensor Peptide MW (g/mol)	Enzyme Used in Sensor Peptide Development	Continuous Assay Strength*
*See tables 2-5 below for Assay Strength definition and reaction conditions.						
CDC25A	cell division cycle 25A	Dual (Y)	CSPS-AQT0523B CSPS-AQT0523K	1189.2	SignalChem (C04-20G)	W
CDC25B	cell division cycle 25B	Dual (Y)	CSPS-AQT0523B CSPS-AQT0523K	1189.2	SignalChem (C04-20BG)	W
CDC25C	cell division cycle 25C	Dual (Y)	CSPS-AQT0523B CSPS-AQT0523K	1189.2	SignalChem (C04-20CG)	W
DUSP22 (JSP-1, LMW-DSP2, MKPX)	dual specificity phosphatase 22	Dual	CSPS-AQT0737B CSPS-AQT0737K	1557.8	SignalChem (D22-20G)	S
DUSP3 (VHR)	dual specificity phosphatase 3	Dual (Y)	CSPS-AQT0264B CSPS-AQT0264K	2004.1	R&D Systems (1654-VH-050)	M
PHLPP1 (PHLPP, SCOP)	PH domain and leucine rich repeat protein phosphatase 1	S/T	CSPS-AQT0481B CSPS-AQT0481K	2110.2	Academic Lab	M
PHLPP2 (PHLPL)	PH domain and leucine rich repeat protein phosphatase 2	S/T	CSPS-AQT0481B CSPS-AQT0481K	2110.2	Academic Lab	M
PPM1A (PP2Ca, PP2CA)	protein phosphatase, Mg2+/Mn2+ dependent 1A	S/T	CSPS-AQT0267B CSPS-AQT0267K	1219.3	SignalChem (P02-20G)	VS
PPM1D (WIP1, PP2C-delta)	protein phosphatase, Mg2+/Mn2+ dependent 1D	S/T	CSPS-AQT0525B CSPS-AQT0525K	2479.5	SignalChem (P04-20G)	S
PPM1G (PP2Cg)	protein phosphatase, Mg2+/Mn2+ dependent 1G	S/T	CSPS-AQT0267B CSPS-AQT0267K	1219.3	SignalChem (P07-20G)	S
PPM1F (CamKpase)	protein phosphatase, Mg2+/Mn2+ dependent 1F	S/T	CSPS-AQT0523B CSPS-AQT0523K-1	1189.2	SignalChem (P06-34G)	M <sup>1</sup>
PPP1CA (PP1A/PPP1A)	protein phosphatase 1 catalytic subunit alpha	S/T	CSPS-AQT0480B CSPS-AQT0480K	2212.3	BPS (41106)	M
PPP1CB (PP1B, PPP1B)	protein phosphatase 1 catalytic subunit beta	S/T	CSPS-AQT0523B CSPS-AQT0523K	1189.2	SignalChem (P14-20G)	VS
PPP2CA (PP2A-α, RP-C)	protein phosphatase 2 catalytic subunit alpha	S/T	CSPS-AQT0523B CSPS-AQT0523K	1189.2	BPS (30056)	VS
PPP2CB (PP2Abeta)	protein phosphatase 2 catalytic subunit beta	S/T	CSPS-AQT0523B CSPS-AQT0523K	1189.2	SignalChem (P17-34G)	W
PPP3CA/B (Calcineurin A/B, CNA alpha/beta, CALNA/B)	protein phosphatase 3 catalytic subunit alpha/beta	S/T	CSPS-AQT0670B CSPS-AQT0670K-1	2529.8	R&D Systems (3160-CA20)	S <sup>1</sup>
PTPN1 (PTP1B, PTB1B)	protein tyrosine phosphatase non-receptor type 1	Y	CSPS-AQT0266B CSPS-AQT0266K	2151.8	BPS (30010)	VS
PTPN2 (TCPTP, PTPT)	protein tyrosine phosphatase non-receptor type 2	Y	CSPS-AQT0264B CSPS-AQT0264K	2004.1	BPS (30013)	VS
PTPN4 (MEG1)	protein tyrosine phosphatase non-receptor type 4	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (30015)	VS
PTPN5 (STEP)	protein tyrosine phosphatase non-receptor type 5	Y	CSPS-AQT0664B CSPS-AQT0664K	1621.6	Academic Lab	M
PTPN6 (SHP-1, PTP-1C)	protein tyrosine phosphatase non-receptor type 6	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (100342)	VS
PTPN7 (HEPTP, LC-PTP)	protein tyrosine phosphatase non-receptor type 7	Y	CSPS-AQT0772B CSPS-AQT0772K	1511.5	SignalChem (P34-20G)	VS
PTPN9 (MEG2)	protein tyrosine phosphatase non-receptor type 9	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (30020)	VS
PTPN11 (SHP-2, PTP2C, PTP1D)	protein tyrosine phosphatase non-receptor type 11	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (30022)	VS
PTPN12 (PTP-PEST, PTPG1)	protein tyrosine phosphatase non-receptor type 12	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (30023)	VS

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PTPN13 (FAP-1, PTP-BAS, PTP-E1, PNP1)	protein tyrosine phosphatase non-receptor type 13	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	SignalChem (P40-21G)	VS
PTPN22 (PTPN8, Lyp)	protein tyrosine phosphatase non-receptor type 22	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	Creative Biomart (PTPN22-01H)	S
PTPRA (R-PTP-alpha, PTPA, LRP)	protein tyrosine phosphatase receptor type A	Y	CSPS-AQT0667B CSPS-AQT0667K	2465.4	SignalChem (P48-21G)	VS
PTPRB (R-PTP-beta, VE-PTP)	protein tyrosine phosphatase receptor type B	Y	CSPS-AQT0667B CSPS-AQT0667K	2465.4	SignalChem (P49-21G)	M
PTPRC (L-CA, CD45, T200)	protein tyrosine phosphatase receptor type C	Y	CSPS-AQT0630B CSPS-AQT0630K	1280.3	BPS (30044)	VS
PTPRE (R-PTP-epsilon)	protein tyrosine phosphatase receptor type E	Y	CSPS-AQT0667B CSPS-AQT0667K	2465.4	SignalChem (P52-21G)	S
PTPRF (LAR)	protein tyrosine phosphatase receptor type F	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	SignalChem (P53-21G)	VS
PTPRG (R-PTP-gamma)	protein tyrosine phosphatase receptor type G	Y	CSPS-AQT0667B CSPS-AQT0667K	2465.4	BPS (30047)	VS
PTPRJ (R-PTP-eta, DEP1, CD148)	protein tyrosine phosphatase receptor type J	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	SignalChem (P56-21G)	VS
PTPRM (PTPμ, PTPRL1, R-PTP-mu)	protein tyrosine phosphatase receptor type M	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	BPS (30053)	VS
PTPRS (R-PTP-sigma)	protein tyrosine phosphatase receptor type S	Y	CSPS-AQT0737B CSPS-AQT0737K	1557.8	SignalChem (P64-21G)	VS

**Note:** Substrate concentrations range from 10-15μM

**Table 1.**

Total Number of PhosphoSens Continuous FI Kinase Assays
Total Number of Assays: 36*
*Please inquire if your target is not listed in the table above.

**Table 2.**

Definition of PhosphoSens Sensor Peptide Assay Strength			
Continuous (FI) Assay strength is defined by Initial Reaction Rate (RFU/pmole/min)			
Very Strong (VS) - >1,000	Strong (S) - 300-999	Medium (M) - 100-299	Weak (W) - 30-99

Table 2.

General Phosphatase Assay Reaction Conditions*
10-15 $\mu$ M PhosphoSens Sensor Peptide
54 mM HEPES, pH 7.5
1.2 mM DTT
0.012% Brij-35
0.5% glycerol
0.22 mg/ml BSA
0.55 mM EGTA
10 mM MgCl <sub>2</sub>
Any additional co-factors or additives (as indicated in Table 4.)

\*Does not account for Enzyme Storage Buffer contributions

Table 3.

Co-factors or Additives*
1. (-) EGTA (+) 5 ng/ $\mu$ l CaM, 0.4 mM CaCl <sub>2</sub>

\*The number designations after the Sensor Peptides Product Numbers denote the co-factors or additives captured above.

Table 4.

General Phosphatase Assay Reaction Set-Up
using a 384-well plate with 25 $\mu$ L total volume in one well at 10 $\mu$ M Sensor Peptide
Add 2 $\mu$ L 100 $\mu$ M Sensor Peptide (CSPS-AQTxxxx)
Add 14 $\mu$ L Master Mix (with Enzyme Reaction Buffer, DTT, and EGTA)
Incubate Reaction Mix (as prepared above) for 5 minutes @ 30°C
Add 4 $\mu$ L Enzyme Dilution Buffer (EDB) (5x) or Enzyme dilutions (5x in EDB)
Put the plate in the reader at 30°C and measure kinetic fluorescent intensity (FI) reading (lExMax 360nm/lEmMax ~492 nm [485-498 nm]) until reaction is no longer linear.

Table 5.

PhosphoSens-Kinetic Phosphatase Kit Components				
Product Number	Component	Description	Volume	Storage
CSPS-AQTxxxxK*	PhosphoSens Substrate, 1 mM	PhosphoSens Sensor Peptide Substrate, 1 mM	35 $\mu$ L	-20°C or below
	DTT Solution, 1 M	1 M DTT in nuclease-free water	150 $\mu$ L	-20°C or below. Minimize repeated freeze/thaw cycles
	Enzyme Reaction Buffer, 10X	500 mM HEPES, pH 7.5, 0.1% Brij-35, 100 mM MgCl <sub>2</sub>	1,650 $\mu$ L	-20°C or below
	Enzyme Dilution Buffer, 5X	20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 1 mg/mL BSA	1,650 $\mu$ L	-20°C or below. Minimize repeated freeze/thaw cycles
	EGTA Solution, 550 mM	550 mM EGTA in 2 M NaOH	150 $\mu$ L	-20°C or below

\*PLEASE NOTE: Any kits with a number designation after 'K-' come with the necessary additives.

Table 6.

PhosphoSens Buffers and Reagents				
Product Number	Component	Description	Volume	Storage
AQT1000XDTT	DTT Solution, 1 M	1 M DTT in nuclease-free water	0.15mL	-20°C or below. Minimize repeated freeze/thaw cycles
AQT10XERB	Enzyme Reaction Buffer, 10X	500 mM HEPES, pH 7.5, 0.1% Brij-35, 100 mM MgCl <sub>2</sub>	15mL	-20°C or below
AQT5XEDB	Enzyme Dilution Buffer, 5X	20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 1 mg/mL BSA	35mL	-20°C or below. Minimize repeated freeze/thaw cycles
AQT1000XEGTA	EGTA Solution, 550 mM	550 mM EGTA in 2 M NaOH	0.15mL	-20°C or below