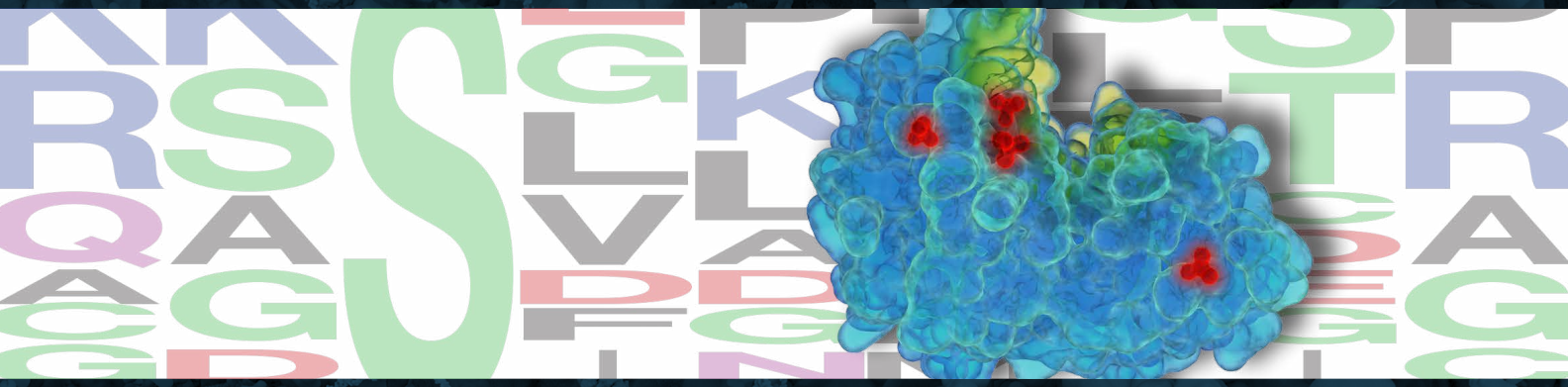


Simplifying Proteomics

PTMScan® Products and Services

Cell Signaling TECHNOLOGY®

Simplifying Proteomics

A limited number of genes can generate a tremendous level of complexity at the protein level due to processes such as alternative splicing and post-translational modification (PTM). PTMs are essential for many cellular functions such as protein activity, subcellular localization, degradation, and protein-protein interactions. Proteomic methods that profile PTMs provide insight into both normal and disease biology that is not feasible at the genetic level.

There are many types of PTMs including:

- Phosphorylation
- Acetylation
- Ubiquitination
- Methylation
- Succinylation
- Proteolytic cleavage

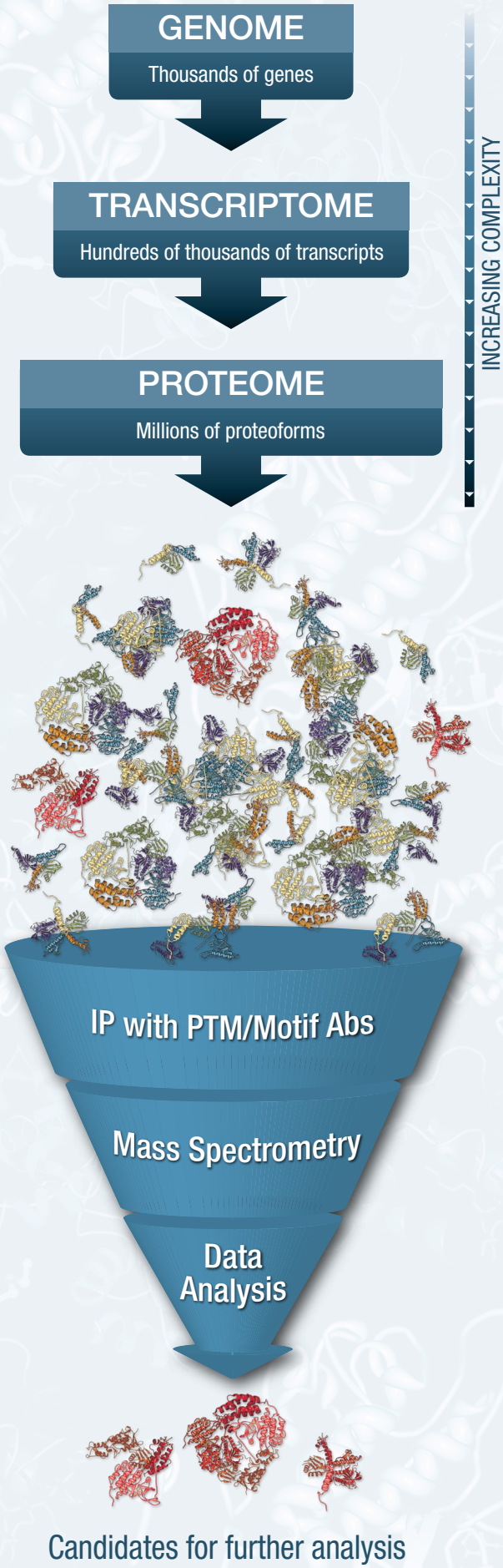
PTMScan®: Antibody enrichment of modified peptides for mass spectrometry-based proteomics

Cell Signaling Technology (CST™) has established PTMScan® technology, a proprietary proteomic method that employs validated PTM- and motif-specific antibodies developed by CST to enrich PTM-containing peptides prior to liquid chromatography tandem mass spectrometry (LC-MS/MS). PTMScan technology allows identification and quantification of hundreds to thousands of even low abundance PTM sites, which can then be narrowed down to the most relevant actionable targets. PTMScan technology uses a more focused approach to PTM-peptide enrichment than other strategies such as immobilized metal affinity chromatography (IMAC).

PTMScan can be used to:

- Determine novel PTM sites that are phosphorylated, ubiquitinated, acetylated, etc.
- Identify and validate drug targets
- Discover biomarkers
- Elucidate off-target drug effects
- Explore the mechanism of action of drugs/chemical modulators

Contents

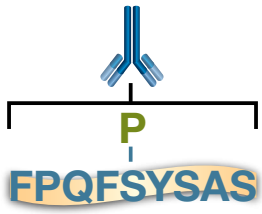
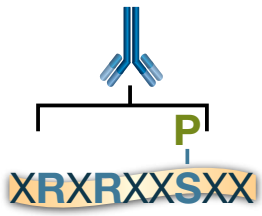
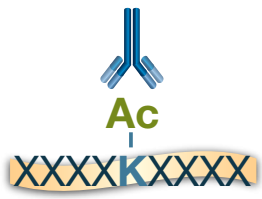


Key to Success: PTMScan® Antibodies

The antibodies used to enrich PTM-containing peptides are key to the success of PTMScan technology. They are:

- Designed and produced in-house
- Rigorously tested for specificity, sensitivity, and lot-to-lot consistency
- Specially formulated for immunoaffinity enrichment

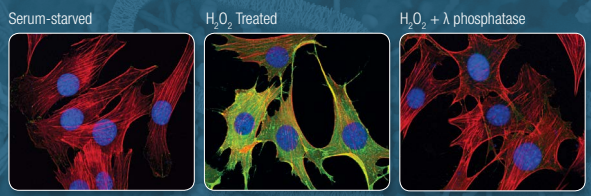
The table below outlines the three types of antibodies for PTMScan technology.

Antibody type	Recognizes	Example	
Standard site-specific PTM antibody	Modified amino acid in the context of a specific sequence of amino acids surrounding it.	A CST™ antibody to Akt1 phosphorylated at serine 473 only recognizes that particular phosphoserine and the surrounding amino acids.	
Motif antibody	Modified amino acid within a certain motif.	The Akt substrate motif antibody will recognize the sequence RXRXXS* in any protein only when the serine residue is phosphorylated (where X can be any amino acid).	
PTM-specific antibody (PTM-antibody)	Any peptide with the PTM of interest.	A CST acetyl-lysine antibody will recognize all acetylation sites independent of flanking amino acid sequences.	

MultiMab™ Antibody Mixtures for Broader Coverage

MultiMab™ rabbit monoclonal antibody mixtures combine individual rabbit monoclonal clones in optimized ratios for broadest possible coverage of the motif/PTM of interest. MultiMab mixtures are used in PTMScan Services and Kits and are available off-the-shelf for other applications.

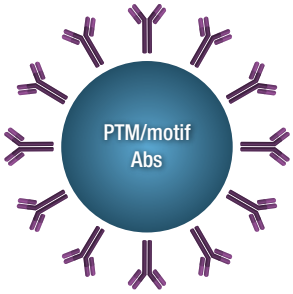
For full list of MultiMab antibody mixtures visit:
www.cellsignal.com/MultiMab



Phospho-Tyrosine (P-Tyr-1000) MultiMab™ Rabbit mAb mix #8954: Confocal IF analysis of C2C12 cells, serum-starved (left), treated with H_2O_2 (2 mM, 10 min; middle), or treated with H_2O_2 followed by λ phosphatase (right), using #8954 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudo-color = DRAQ5® #4084 (fluorescent DNA dye).

Discovery vs. Direct

Which option is right for your research?

	PTMScan® Discovery (PTM/motif-based enrichment)	PTMScan® Direct (mass spectrometry-based antibody array)
		
Is a specific pathway targeted?	✘	✔
Is antibody enrichment performed?	✔	✔
Is LC-MS/MS performed?	✔	✔
What type of antibodies are used?	PTM or motif antibodies to undefined targets.	Standard site-specific antibodies to defined targets within the known pathway(s) of interest.
What is the bead format?	Antibodies against one PTM or motif on each bead.	Antibodies against many targets on each bead (a bead-based multiplex assay).
Which species can you use?	Can be used on samples from many different species including, but not limited to, human, mouse, rat, drosophila, and arabidopsis.	Validated for human and mouse. (Contact us for other species.)
Case Study	"Deep, quantitative coverage of the acetylome using novel anti-acetyl-lysine antibodies and an optimized proteomic workflow." Svinkina, T., et al (2015) *Mol. Cell. Proteomics* 14(9):2429–40.	"PTMScan Direct: identification and quantification of peptides from critical signaling proteins by immunoaffinity enrichment coupled with LC-MS/MS." Stokes, M., et al (2012) *Mol Cell Proteomics.* 11(5):187–201.
Summary	**Use PTMScan Discovery to find new information with quantitative analysis of PTMs.**	**Use PTMScan Direct to quantitatively assay the activity of components of known signaling pathways across cell lines or treatments.**

PTMScan® Kits vs. Services

PTMScan® Kits

With PTMScan® Antibody Kits you can perform your own enrichment and LC-MS/MS analysis. They provide the antibody reagents for 10* peptide enrichment experiments and the detailed protocols needed to discover new sites of post-translational modification.§

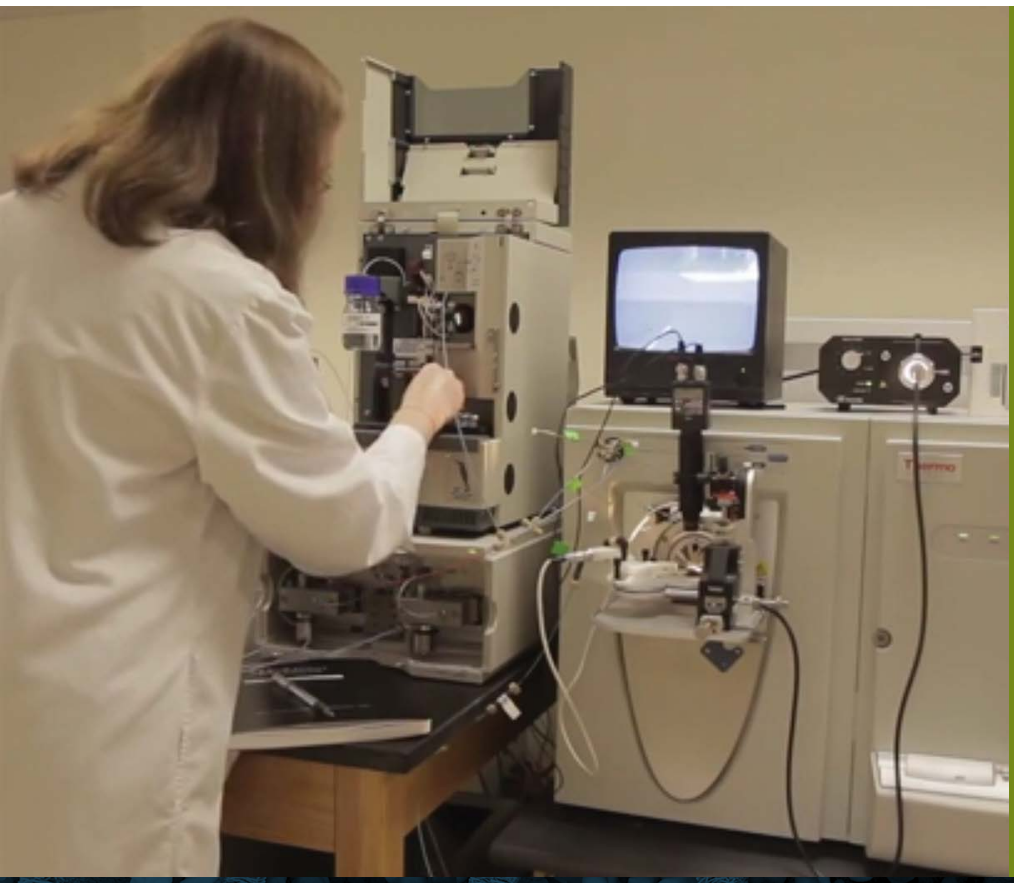
*A smaller format for certain kits (3 assays instead of 10) allows investigators to run pilot studies.

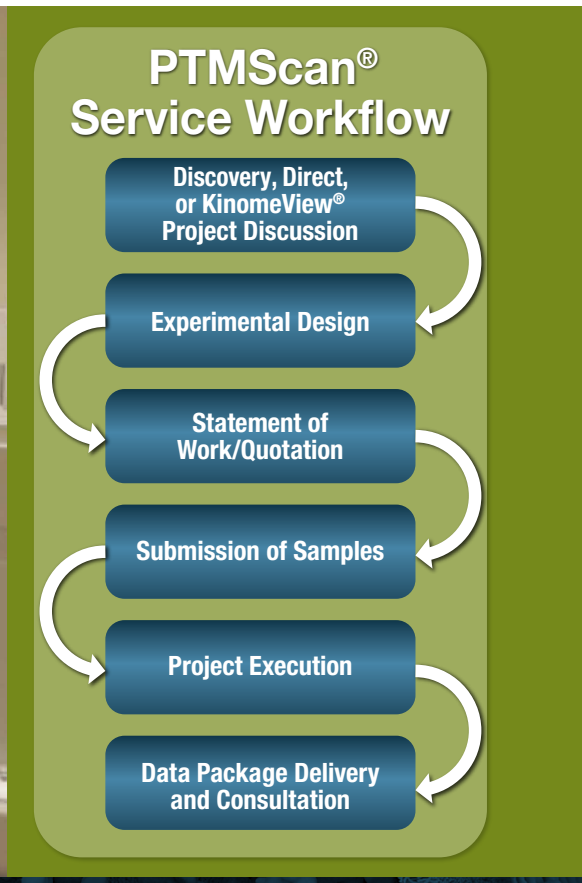
§Kits also contain a limited use license.

PTMScan® Services

CST scientists work with you from project planning to delivery of a comprehensive data package that includes:

- Qualitative/quantitative tables
- Informatics tables
- Microsoft® PowerPoint® summary
- Microsoft® Word® guide to prioritizing follow-up candidates



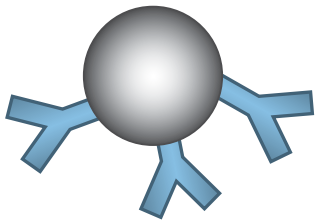


PTMScan® Discovery and Direct Services

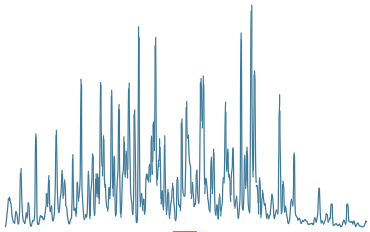
Workflow

Protease-digested Sample

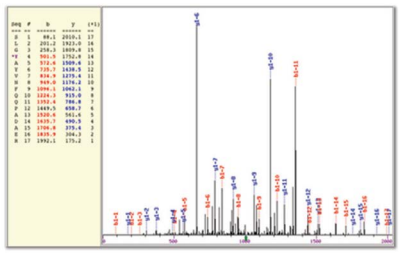
PTMScan® Reagent Immobilized to Protein-A or -G Agarose



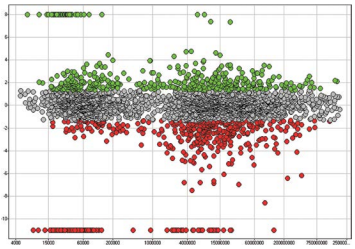
Analyze Eluted Peptide Fraction by LC-MS/MS

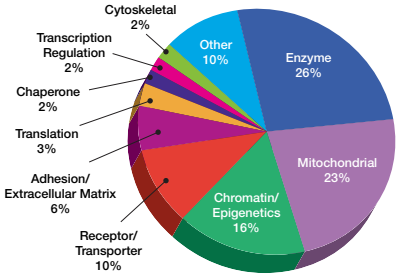


Assign Sequences to MS/MS Spectra with SEQUEST®



Relative Quantification/Data Analysis





PTMScan® Service Data

The data set generated by a PTMScan® service experiment includes quantification of PTM changes, the identity of each protein, and specific location of each modification site.

Normalized Fold Change						
SU11274 vs. DMSO Control	Staurosporine vs. DMSO Control	Protein Name	Site	-7/+ 7 Sequence	Peptide	Upstream Kinase
-5.0	-4.6	EphA2	897	RVSIRLPsTSGSEGV	LPS*T*SGSEGVPFR	Akt1
-13.6	-2.1	FOXO1A	319	TFRPRTSsNASTISG	TSS*NASTISGR	Akt1
-158.0	-7.2	FOXO4	32	QSRPRSCtWPLPRPE	SCT*WPLPRPEIANQPSEPPEVEPDLGEK	Akt1
-3.4	1.8	QIK	358	DGRQRRPsTIAEQTV	RPS*TIAEQTVAK	Akt1, Akt2
-13.3	-29.4	S6	235, 236, 240	IAKRRRLsSLRASTS	RLS*S*LRAS*TSK	Akt1, Akt2, P70S6Kβ, PKACα, PKCα, PKCδ
-7.0	-24.5	S6	236, 240	AKRRRLSsLRASTSK	RLSS*LRAS*TSK	Akt1, Akt2, P70S6Kβ, PKACα, PKCα, PKCδ
2.6	1.1	BRAF	365	GQRDRSSsAPNVHIN	SSS*APNVHINTIEPVNIDDLIR	Akt1, Akt3
-7.0	-9.4	GSK3β	9	SGRPRTTsFAESCKP	TTS*FAESCKPVQQPSAFGSMK	Akt1, AurA, CAMK2β, GSK3β, KHS1, PKACα, PKCα
-5.3	-N.D.	GSK3β	9, 21	SGRPRTTsFAESCKP	TTS*FAESCKPVQQPS*AFGSMK	Akt1, AurA, CAMK2B, GSK3β, KHS1, PKACα, PKCα
-21.3	-3.0	PEA-15	116	KDIIRQPsEEEIIKL	DIIRQPS*EEEIIK	Akt1, CAMK2α, CK2α1
-2.1	-2.9	GSK3α	21	SGRARTSsFAEPGGG	TSS*FAEPGGGGGGGGGPGGSASGPGGTGGGK	Akt1, CAMK2β, PKACA, PKCα, PKCβ
-10.3	-1.8	RANBP3	126	VKRERTSsLTQFPPS	TSS*LTQFPPSQSEER	Akt1, ERK1, RSK2, p90RSK
2.7	2.5	eIF4B	422	RERSRTGsESSQTGT	TGS*ESSQTGTSTTSSR	Akt1, p70S6K, p90RSK
4.8	2.5	eIF4B	422, 425	RERSRTGsESSQTGT	TGS*ESS*QTGTSTTSSR	Akt1, p70S6K, p90RSK

Table view presentation of data from PTMScan® analysis of MKN-45 cells treated with SU11274 or staurosporine. Shown are representative data for the basophilic Akt substrate motifs RXRXX(s/t) and RXX(s/t). Relative abundance changes of 2.5-fold or greater (treated versus control) for phosphorylated peptides are indicated by green (increase) or red (reduction) highlighting.

Complementary Services

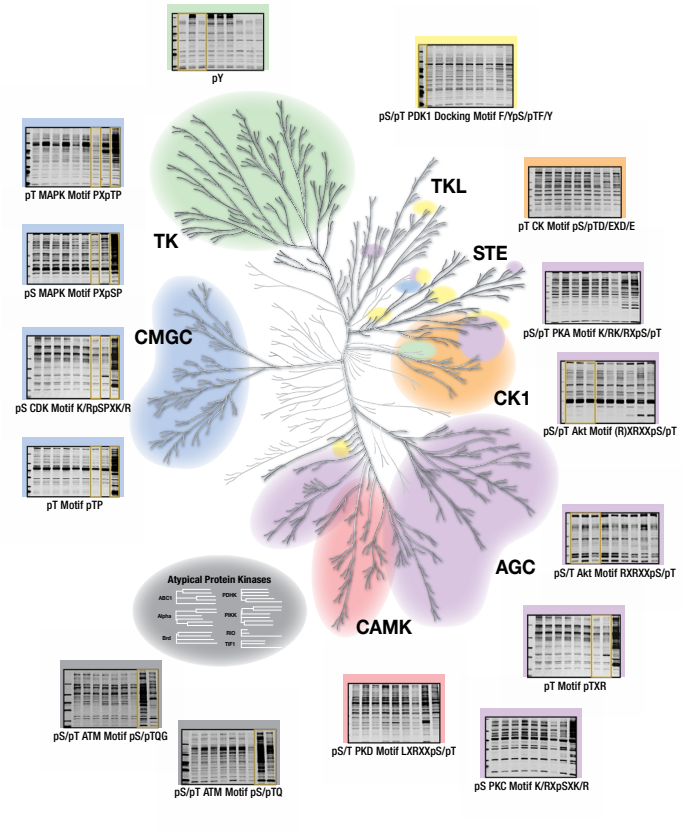
KinomeView® Western Blot Profiling

KinomeView® profiling is a western blot prescreen using the same PTM and motif antibodies available for PTMScan analysis.

- Observe global signaling changes across multiple samples
- Select the best antibodies for use in subsequent PTMScan Discovery experiments
- Optimize experimental conditions (treatment dose, time point, cell line or tissue, etc.)

The KinomeView® Profiling Kit #9812 or Service

The kit provides motif antibodies for a kinome-wide view of cellular phosphorylation for customers who want to perform the experiment themselves. The KinomeView® Profiling Service also includes motif antibodies for studying changes in additional PTMs. The service is performed by CST scientists and includes data analysis and expert project consultation.



Immobilized Metal Affinity Chromatography (IMAC) Service

IMAC is another strategy for enriching phosphorylated peptides before LC-MS/MS. It captures negatively charged phosphopeptides using positively charged metal ions.

Global Proteome Profiling Service

Quantification of protein levels across cell lines or treatments without enrichment for PTM-containing peptides.

Ingenuity® Pathway Analysis

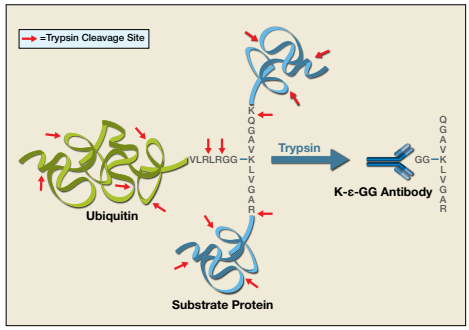
Mapping of PTMScan® results onto canonical pathways and new interaction networks using Qiagen's Ingenuity® Pathway Analysis (IPA) software.

PTMScan® Discovery Products and Services

UbiScan® (Ubiquitination Proteomics)

Protein ubiquitination is involved in many cellular processes including proteasomal degradation, endocytosis, DNA repair, cell cycle regulation, and gene expression. Abnormal ubiquitination is involved in diseases such as cancer, metabolic syndrome, and neurodegenerative diseases.

UbiScan® proteomics uses a proprietary antibody against the di-glycine remnant (K-ε-GG) left on ubiquitinated lysine residues after trypsin digestion. This ubiquitin remnant motif antibody is used to enrich ubiquitinated peptides from a trypsin digested sample prior to LC-MS/MS analysis.



Ubiquitin Remnant Motif (K-ε-GG): The Ubiquitin Remnant Motif (K-ε-GG) antibody is used to enrich K-ε-GG containing peptides from complex mixtures.

UbiScan® – Ubiquitination Proteomics		Antibody‡	PTMScan®		KinomeView®	
Target	Motif	No.	Kit	Service	Kit #9812	Service
Ubiquitin Remnant	K-ε-GG	–	#14482/#5562	✔	–	✔

Select UbiScan® Publications:

Sook-Young, J. et al. (2015) Proteomic Analysis of Ubiquitin-Like Posttranslational Modifications Induced by the Adenovirus E4-ORF3 Protein. *Journal of Virology* 89(3) 1744-55.

Kronke, J. et al. (2015) Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. *Nature* 523(75599), 183–188.

Kronke, J. et al. (2014) Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* 343(6168), 301–305.

Theurillat, J.P. et al. (2014) Ubiquitylome analysis identifies dysregulation of effector substrates in SPOP-mutant prostate cancer. *Science* 346(6205), 85–89.

Mathew, R. et al. (2012) BTB-ZF factors recruit the E3 ligase cullin 3 to regulate lymphoid effector programs. *Nature* 491(7425), 618–621.

Emanuele, M.J. et al. (2011) Global identification of modular Cullin-RING ligase substrates. *Cell* 147(2), 459–474.

Kim, W. et al. (2011) Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44(2), 325–340.

PhosphoScan® (Phosphorylation Proteomics)

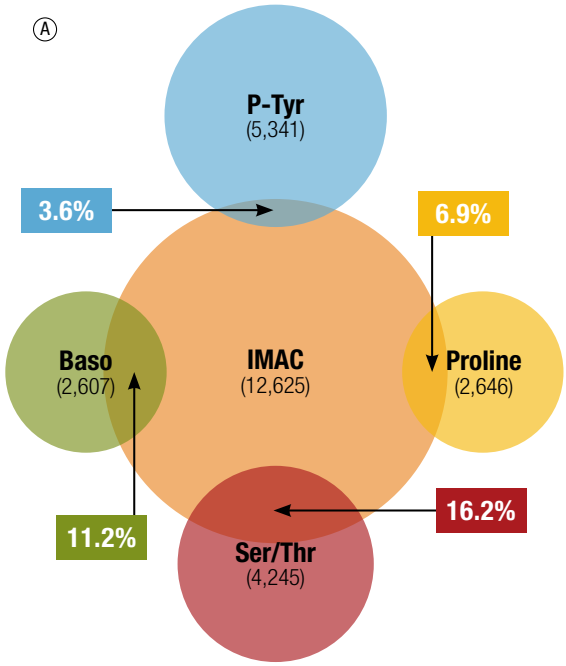
Complementary Phosphopeptide Enrichment Strategies

Comparison of PTMScan® and IMAC enrichment methods: PTM profiling of human gastric carcinoma cells.

Phosphopeptides were enriched using various classes of motif/PTM antibodies (tyrosine, proline, serine/threonine, and basophilic), or IMAC.

- Over 20,000 phosphorylation sites were identified across all samples.
- There was minimal overlap between phosphopeptides enriched using antibody-based versus IMAC methods.
- Antibody-based methods reduce sample complexity for a more focused approach to phosphopeptide enrichment.

Antibody-based and IMAC enrichment strategies are highly complementary and both should be used to get the broadest coverage of the phosphoproteome.



Area proportional Venn diagram showing the overlap between motif antibody and IMAC enrichment of phosphopeptides from MKN-45 cells **(A)**. Comparisons were made on unique proteins/sites. MKN-45 cells were treated and profiled as outlined **(B)**. Combinations of motif antibodies were used to enrich phosphopeptides as indicated **(C)**.

View more data showing complementarity of PTMScan Discovery and IMAC at: **www.cellsignal.com/PTMScanIMAC**

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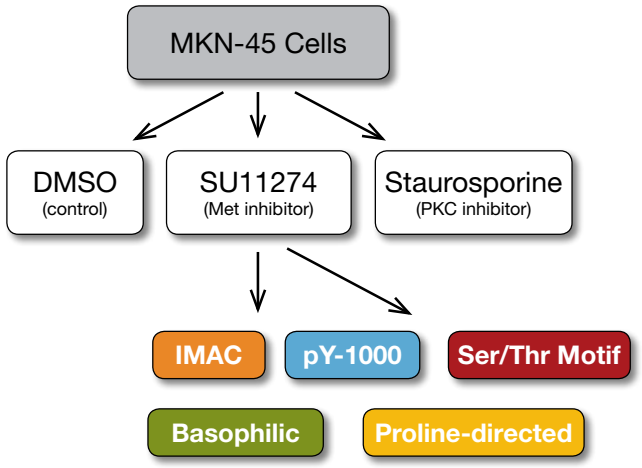


Ⓒ

Antibody	Motif	pY	Baso	Pro	Ser/Thr
Phosphotyrosine	Y	●			
Akt Substrate	RXX(S/T)		●		●
Akt Substrate	RXRXX(S/T)		●		●
AMPK/PKD Substrate	LXRXX(S/T)		●		●
CDK Substrate	(K/R)(S/T)PX(K/R)		●		●
PKA Substrate	(K/R)(K/R)X(S/T)		●		●
PKC Substrate	(K/R)X(S/T)(K/R)		●		●
MAPK Substrate	PX(S/T)P			●	●
PLK Binding Motif	S(S/T)P			●	●
tP Motif	(S/T)P			●	●
tPE Motif	(S/T)PE			●	●
tXR/tPR Motif	(S/T)(X/P)R			●	●
14-3-3 Binding Motif	(R/K)XX(S/T)XP			●	●
ATM/ATR Substrate	(S/T)Q				●
ATM/ATR Substrate	(S/T)QG				●
CK Substrate	(S/T)(D/E)X(D/E)				●

Note: *Custom mixes of Ser/Thr motif antibodies can be made for service projects. Please inquire.*

PhosphoScan® – Phosphorylation Proteomics		Antibody‡	PTMScan®		KinomeView®	
Target	Motif	No.	Kit	Service	Kit #9812	Service
14-3-3 Binding Motif	R/KXXSXP	#9601	–	✔	–	✔
Akt Substrate	RXX(S/T)	#9614	#5561	✔	✔	✔
Akt Substrate	RXRXX(S/T)	#10001	#5563	✔	✔	✔
(Ser/Thr) AMPK Substrate	(L/M)XRXX(S/T)	#5759	#5564	✔	✔	✔
(Ser) ATM/ATR Substrate	SQ	#9607	#12267	✔	✔	✔
(Ser/Thr) ATM/ATR Substrate	(S/T)QG, (S/T)Q	#6966	#12267	✔	✔	✔
(Ser) CDKs Substrate	(K/R)SPX(K/R)	#9477	–	✔	✔	✔
CK2 Substrate	(S/T)DX(D/E)	#8738	#12170	✔	✔	✔
MAPK/CDK Substrate	PXSP and SPX(K/R)	#2325	#4652	✔	✔	✔
PKA Substrate	(K/R)(K/R)X(S/T)	#9624	#5565	✔	✔	✔
(Ser) PKC Substrate	(K/R)XSX(K/R)	#6967	–	✔	✔	✔
(Ser/Thr) PKD Substrate	LXRXX(S/T)	#4381	–	✔	–	✔
(Thr) PLK Binding Motif	STP	#5243	#5566	✔	✔	✔
Thr-Pro-Glu Motif	TPE, TP	#3004	–	✔	✔	✔
Thr-Pro Motif	TP, TPP	#5757	#5567	✔	✔	✔
Thr-X-Arg Motif	TXR, TPR	#2351	–	✔	✔	✔
Tyrosine Rabbit (P-Tyr 1000)	Y	#8954	#14478/#8803	✔	✔	✔

Select PhosphoScan® Publications:

Lee, M.E. et al. (2015) Endothelial Akt1 mediates angiogenesis by phosphorylating multiple angiogenic substrates. *PNAS* 111(35), 12865–12870.

Paardekooper Overman, J. et al. (2014) PZR Coordinates Shp2 Noonan and LEOPARD syndrome signaling in zebrafish and mice. *Mol. Cell. Biol.* 34(15), 2874–2889.

Siddoway, B. et al. (2013) Synaptic activity bidirectionally regulates a novel sequence-specific S-Q phosphoproteome in neurons. *Journal of Neurochemistry* 128(6):841–851.

Ren, H., et al. (2012) Identification of anaplastic lymphoma kinase as a potential therapeutic target in ovarian cancer. *Cancer Res.* 72(13) 3312–3323.

Carretero, J. et al. (2010) Integrative genomic and proteomic analyses identify targets for Lkb1-deficient metastatic lung tumors. *Cancer Cell* 17(6), 547–559.

Moritz, A. et al. (2010) Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. *Sci. Signal* 3(136), ra64.

Rikova, K. et al. (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131(6), 1190–1203.

Griswold, I.J., et al. (2006) Kinase domain mutants of Bcr-Abl exhibit altered transformation potency, kinase activity, and substrate utilization, irrespective of sensitivity to imatinib. *Mol Cell Biol.* 26(16) 6082–6093.

Rush, J. et al. (2005) Immunoaffinity profiling of tyrosine phosphorylation in cancer cells. *Nat. Biotechnol.* 23(1), 94–101.

‡ *Validated for use in other applications.*

PTMScan® Discovery Products and Services (cont.)

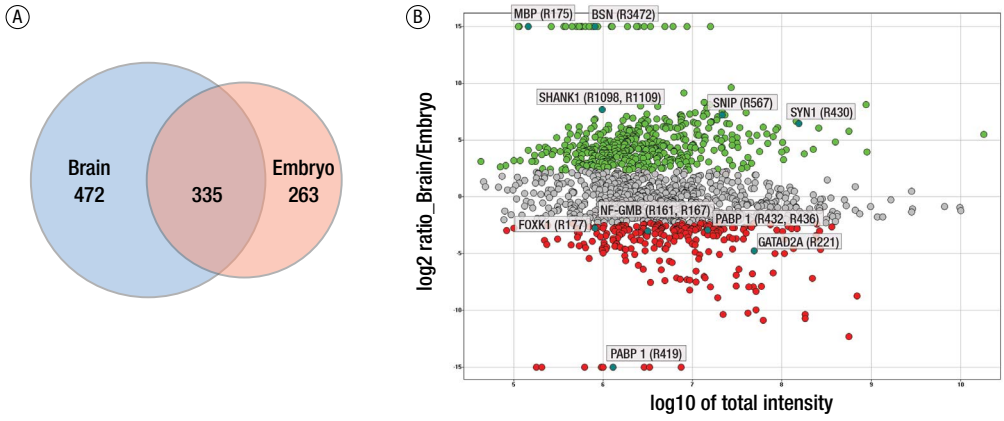
MethylScan® (Methylation Proteomics)

Protein methylation is a common post-translational modification (PTM) that mostly occurs on arginine and lysine residues. Arginine methylation regulates processes such as RNA processing, gene transcription, DNA damage repair, protein translocation, and signal transduction. Lysine methylation is best known to regulate histone function and is involved in epigenetic regulation of gene transcription.

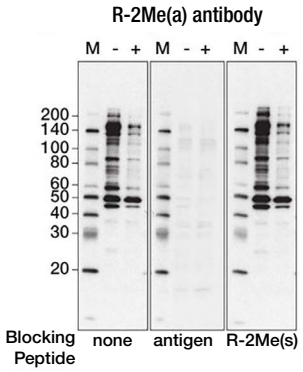
MethylScan® proteomics uses proprietary methyl arginine (Me-R) or methyl lysine (Me-K) antibodies to enrich methyl-containing peptides from trypsin digested samples prior to LC-MS/MS analysis.

Features and Benefits

- PTMScan® methyl antibodies are exceptionally specific, as demonstrated by peptide blocking experiments, to help ensure the most accurate results.
- PTMScan technology can be applied to many biological systems and species to encompass diverse research interests.
- Experienced CST scientists provide technical support throughout the PTMScan workflow to facilitate research progress.



Quantitative analysis of arginine methylation in mouse brain and embryo: Venn diagrams of the number of monomethyl arginine (R-Me) and asymmetric dimethyl arginine (R-2Me(a)) sites identified in mouse brain and embryo **(A)**. Quantitative comparison of arginine monomethylation in the two tissues. Each dot in the scatter plot represents a unique arginine monomethylated peptide identified using PTMScan® Mono-Methyl Arginine Motif [mme-RG] Kit #12235. The x-axis is the log10 value of the total intensity of the representative peptide for a methylation site in mouse brain and embryo, and the y-axis shows the log2 ratio of intensity of the peptide in mouse brain vs. embryo. A cutoff of 5-fold was set to indicate increased arginine monomethylation peptide abundance in either brain (green dots) or embryo (red dots). For the methyl peptides that uniquely existed in a specific tissue, arbitrary log2 ratios of 15 (brain specific) and -15 (embryo specific) were assigned **(B)**.*



Western blot analysis of lysates from untreated (-) or AdOx-treated (+) HCT116 cells using asymmetric dimethyl arginine (R-2Me(a)) antibodies. The specific signal was blocked by antigen R-2Me(a) peptides (middle panel), but not by corresponding symmetric dimethyl arginine (R-2Me(s)) peptides (right panel).*

*This research was originally published in Molecular and Cellular Proteomics. Guo, A., et al. Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. *Mol. Cell. Biol.* 2014; 13(1):372–387.

MethylScan® – Methylation Proteomics		Antibody‡	PTMScan®		KinomeView®	
Target	Motif	No.	Kit	Service	Kit #9812	Service
Mono-Methyl Arginine	R-Me	#8015	#12235	✔	–	✔
Asymmetric Di-Methyl Arginine	R-2Me(a)	#13522	#13474	✔	–	✔
Symmetric Di-Methyl Arginine	R-2Me(s)	#13222	#13563	✔	–	✔
Pan-Methyl Lysine	K-Me/K-2Me/K-3Me	#14117 (2Me) #14680 (3Me)	#14809	✔	–	✔

Select MethylScan® Publications:

Guo, A. et al. (2014) Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. *Mol. Cell. Proteomics* 13(1) 372–387.

Sylvestersen, K. B. et al. (2014) Proteomic analysis of arginine methylation sites in human cells reveals dynamic regulation during transcriptional arrest. *Mol. Cell. Proteomics* 13(8):2072–2088.

‡ *Validated for use in other applications.*

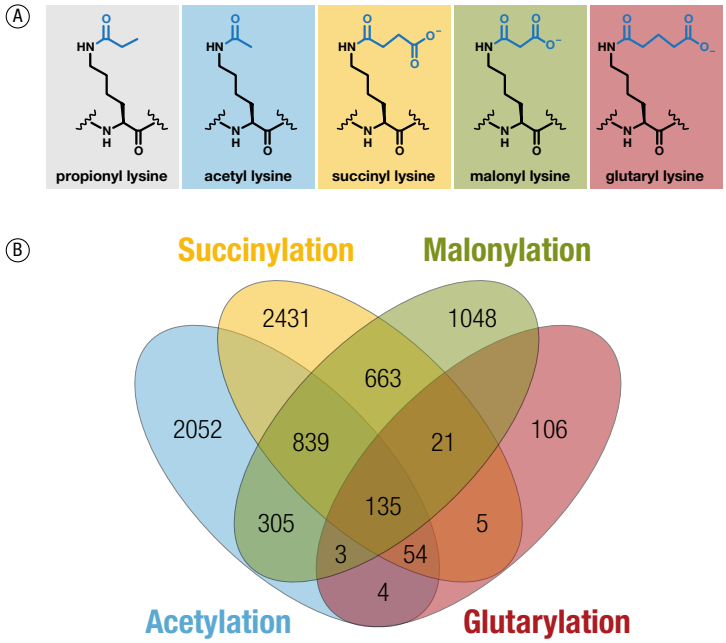
AcylScan™ (Acylation Proteomics)

Lysine residues are subject to a wide range of modifications. Acyl group transfer from the metabolic intermediates acetyl-, succinyl-, malonyl-, glutaryl-, butaryl-, and crotonyl- CoA all neutralize the positive charge of lysine and confer structural alterations affecting substrate protein function.

AcylScan™ proteomics use proprietary acetyl- (Ac-K), glutaryl- (Glut-K), malonyl- (Mal-K), propionyl- (Prop-K), or succinyl-lysine (Succ-K) antibodies to enrich their respective acyl-containing peptides from trypsin digested samples prior to LC-MS/MS analysis.

Features and Benefits

- AcylScan methods allow quantitative profiling of thousands of acetylation, glutarylation, malonylation, propionylation, and succinylation events by using antibodies with exquisite specificity and sensitivity.
- PTMScan technology can be applied to many biological systems and species to encompass diverse research interests.
- Experienced CST scientists provide technical support throughout the PTMScan workflow to facilitate research progress.



Profiling lysine acylation in liver peptides from wild type and Sirt5 knockout mice: Diagram of five acylation PTM types **(A)**. The degree of overlap of sites identified using the four specified acylation-specific antibodies **(B)**.

		Antibody‡	PTMScan®		KinomeView®	
Target	**Motif**	**No.**	**Kit**	**Service**	**Kit #9812**	**Service**
AcetylScan® – Acetylation Proteomics						
Acetyl-Lysine	Ac-K	#9814	#14499/#13416	✔	–	✔
GlutarylScan™ – Glutarylation Proteomics						
Glutaryl-Lysine	Glut-K	–	#26101	✔	–	✔
MalonylScan™ – Malonylation Proteomics						
Malonyl-Lysine	Mal-K	–	#93872	✔	–	✔
PropionylScan™ – Propionylation Proteomics						
Propionyl-Lysine	Prop-K	–	#17848	✔	–	✔
SuccinylScan™ – Succinylation Proteomics						
Succinyl-Lysine	Succ-K	–	#13764	✔	–	✔

Select AcylScan™ Publications:

Svinkina, T. et al. (2015) Deep, quantitative coverage of the lysine acetylome using novel anti-acetyl-lysine antibodies and an optimized proteomic workflow. *Mol. Cell Proteomics* 14(9), 2429–2440.

Bouchut, A. et al. (2015) Proteome-wide lysine acetylation in cortical astrocytes and alterations that occur during Infection with brain parasite toxoplasma gondii. *PLoS ONE* 10(3), e0117966.

Ryder, D.J. et al. (2015) Identification of the acetylation and ubiquitin-modified proteome during the progression of skeletal muscle atrophy. *PLoS ONE* 10(8), e0136247.

Rardin , M.J. et al. (2013) SIRT5 regulates the mitochondrial lysine succinylome and metabolic networks. *Cell Metab.* 18(6), 920–933.

Mielcarek, M. et al. (2013) HDAC4 Does Not Act as a Protein Deacetylase in the Postnatal Murine Brain In Vivo. *PLoS One* 8(11) e80849.

Jeffers, V. et al (2012) Lysine acetylation is widespread on proteins of diverse function and localization in the protozoan parasite Toxoplasma gondii. Eukaryot. *Cell* 11(6) 735–742.

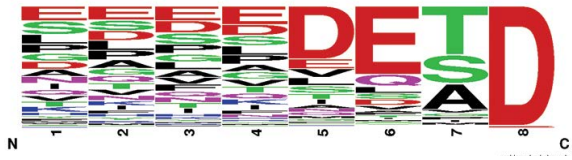
‡ *Validated for use in other applications.*

PTMScan® Discovery Products and Services (cont.)

Caspase Cleavage Substrate Proteomics

The intrinsic and extrinsic apoptotic pathways involve a cascade of caspases. The human proteome contains thousands of known or putative caspase cleavage sites. The majority of caspase substrates are cleaved at an aspartic acid residue, generating fragments containing a carboxy-terminal aspartate with a general DEXD motif.

PTMScan® technology for caspase cleavage substrate proteomics uses an antibody to the DEXD motif to enrich even low abundance cleaved caspase substrate peptides from trypsin digested samples prior to LC-MS/MS analysis.



Caspase Cleavage Substrate Proteomics: The motif logo was generated from a PTMScan® LC-MS/MS experiment using 1044 nonredundant tryptic peptides with carboxy-terminal aspartates derived from HeLa cells treated with Staurosporine #9953 (1 µM, 3 hr) to induce apoptosis. Peptides were enriched using the PTMScan® Cleaved Caspase Substrate Motif [DE(T/S/A)D] Kit #12810. The logo represents the relative frequency of amino acids in each position leading up to the carboxy-terminal aspartate.

Features and Benefits

- The proprietary cleaved caspase substrate motif antibody is a unique and sensitive tool for quantitative profiling of hundreds to thousands of caspase cleaved substrates.
- PTMScan technology can be applied to many biological systems and species to encompass diverse research interests.
- Experienced CST scientists provide technical support throughout the PTMScan workflow to facilitate research progress.

Caspase Cleavage Substrate Proteomics

		Antibody‡	PTMScan®		KinomeView®	
Target	Motif	No.	Kit	Service	Kit #9812	Service
Caspase Cleavage Substrate	[DE(T/S/A)D]	#8698	#12810	✔	–	✔

‡ *Validated for use in other applications.*

Select Caspase Cleavage Substrate Publications:

Anania, V.G. and Lill, J.R. (2015) Proteomic tools for the characterization of cell death mechanisms in drug discovery. *PROTEOMICS - Clinical Applications* 9(7-8) 671–683.

Pham, V.C. et al. (2012) Complementary proteomic tools for the dissection of apoptotic proteolysis events. *J. Proteome Res.* 11(5), 2947–2954.

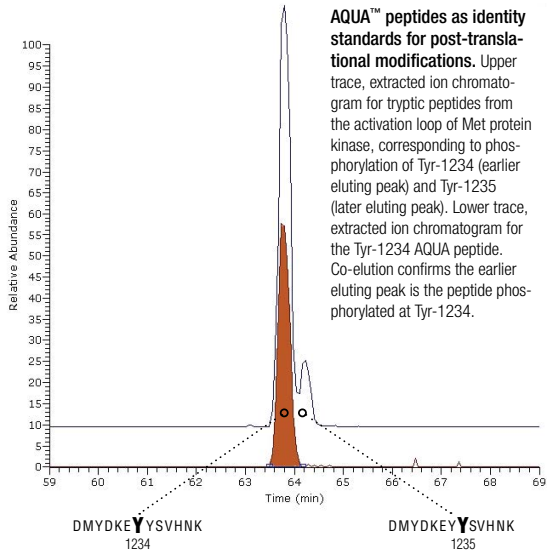
PTMScan® Companion Products

To support your proteomics experiments, we offer the following fully validated companion reagents:

- **Mouse Control Peptides** for ensuring run-to-run performance of the LC-MS/MS system.
- **Immunoaffinity Purification (IAP) Buffer** for reconstitution of lyophilized peptides prior to immunoaffinity purification.
- **AQUA™ Peptides*** for validating and quantifying protein markers and post-translational modifications.

PTMScan® Companion Products

Product	No.
PTMScan® Trypsin Digested Control Peptides I	#12219
PTMScan® Lys-C Digested Control Peptides I	#12148
PTMScan® IAP Buffer (10X)	#9993



AQUA™ peptides as identity standards for post-translational modifications. Upper trace, extracted ion chromatogram for tryptic peptides from the activation loop of Met protein kinase, corresponding to phosphorylation of Tyr-1234 (earlier eluting peak) and Tyr-1235 (later eluting peak). Lower trace, extracted ion chromatogram for the Tyr-1234 AQUA peptide. Co-elution confirms the earlier eluting peak is the peptide phosphorylated at Tyr-1234.

*This method was developed by scientists at Harvard Medical School. Limited use of this method is permitted under a licensing arrangement with Harvard College.

For more information about AQUA peptides and other companion products visit: **www.cellsignal.com/PTMScancompanion**

PTMScan® Direct Services

CST offers six PTMScan® Direct services that can be used to investigate changes in PTMs of specific or known protein targets in response to a drug treatment or disease state.

	Number of unique PTM sites*	Number of Proteins
PTMScan® Direct Multi-Pathway	1,006	409
PTMScan® Direct PI3K / Akt Signaling	296	105
PTMScan® Direct Apoptosis & Autophagy	175	100
PTMScan® Direct Cell Cycle & DNA Damage	263	168
PTMScan® Direct Ser/Thr Kinases	385	130
PTMScan® Direct Tyrosine Kinases	671	120

* For a full list of targets in each PTMScan Direct reagent visit: **www.cellsignal.com/PTMScanDirect**.

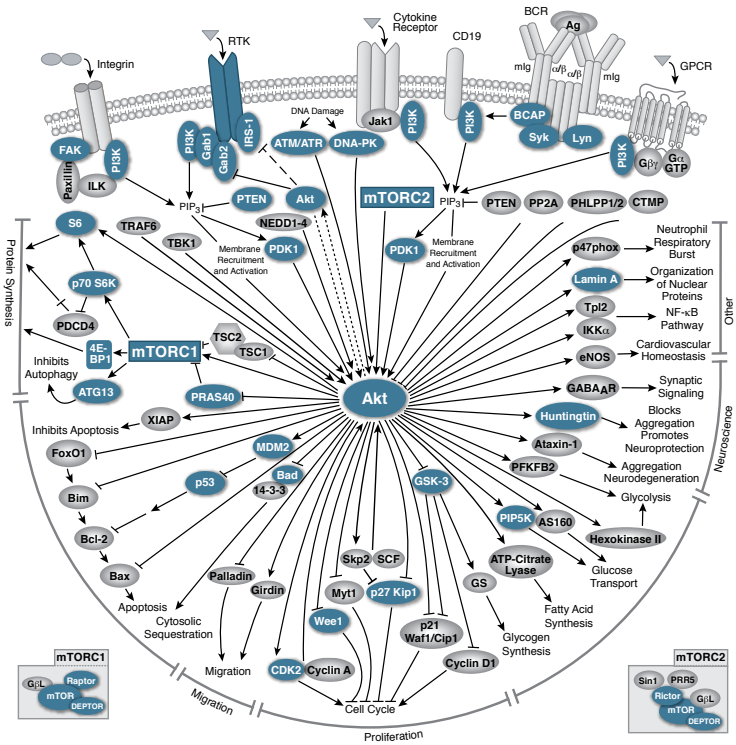
PTMScan® Direct Multi-Pathway

PTMScan® Direct Multi-Pathway Service quantitatively measures a defined set of peptides spanning a large number of signaling pathways, including:

- Adherens Junction Dynamics
- AMPK Signaling
- B Cell Receptor Signaling
- Cell Cycle Control: G1/S Checkpoint
- Cell Cycle Control: G2/M DNA Damage Checkpoint
- ErbB/HER Signaling
- G Protein-coupled Receptor Signaling to MAPK/Erk
- Insulin Receptor Signaling
- Jak/Stat Signaling: IL-6 Receptor Family
- MAPK Signaling Cascades
- MAPK/Erk in Growth and Differentiation
- NF-κB Signaling
- PI3 Kinase/Akt Signaling*
- Regulation of Actin Dynamics
- Regulation of Microtubule Dynamics
- SAPK/JNK Signaling Cascades
- Signaling Pathways Activating p38 MAPK
- T cell Receptor Signaling
- Toll-like Receptor Signaling

**The PI3 Kinase/Akt Signaling pathway is just one pathway covered by the PTMScan Direct Multi-pathway reagent.*

NOTE: Highlighted **blue** nodes designate proteins within the PI3K/Akt pathway with peptides targeted by the PTMScan® Direct Multi-Pathway Service.



Select PTMScan® Direct Publications:

Stokes, M.P. et al. (2013) Quantitative profiling of DNA damage and apoptotic pathways in UV damaged cells using PTMScan Direct. *Int. J. Mol. Sci.* 14(1), 286–307.

Pease, B.N. etal. (2013) Global Analysis of Protein Expression and Phosphorylation of Three Stages of Plasmodium falciparum Intraerythrocytic Development. *J. Proteome Res.* 2(9):4028–4045.

Stokes, M.P. et al. (2012) PTMScan Direct: identification and quantification of peptides from critical signaling proteins by immunoaffinity enrichment coupled with LC-MS/MS. *Mol. Cell Proteomics* 11(5), 187–201.

Post-PTMScan® Validation Studies

CST products to follow-up on candidates identified in PTMScan® Discovery or PTMScan® Direct include:

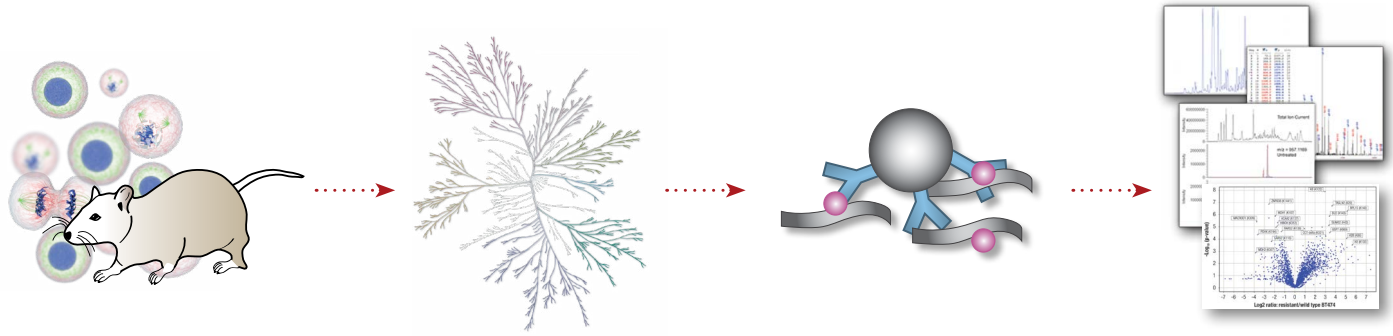
- Modification state-specific and total protein antibodies produced and validated in-house for multiple applications
- SignalSilence® siRNA for knockdown of specific human or mouse proteins
- PathScan® Sandwich ELISA Kits and Antibody Pairs for analysis of a large selection of intracellular molecules
- AQUA™ peptides for multiple reaction monitoring (MRM) or immuno-MRM mass spectrometry assays

From Bench to Bedside

PTMScan® Technology in Translational Research

PTMScan® Discovery workflow for phospho-tyrosine modifications altered in NSCLC

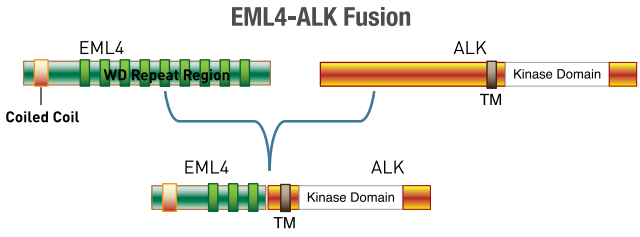
CST performed a global survey of tyrosine kinase activity in non-small cell lung cancer (NSCLC) to identify novel disease drivers **(1)**. The PTMScan® Phospho-Tyrosine Mouse mAb (P-Tyr-100) kit was used to enrich phosphorylated peptides from 41 NSCLC cell lines and 150 NSCLC tumors. This analysis identified 4,551 phospho-tyrosine residues on more than 2,700 proteins. The tyrosine kinase ALK (anaplastic lymphoma kinase) was among the top 10 candidates for follow up analysis.



Cell or Tissue Samples | Kinase Family Targeting | Immunoprecipitation Using Motif Antibody | LC-MS/MS and Bioinformatic Analysis

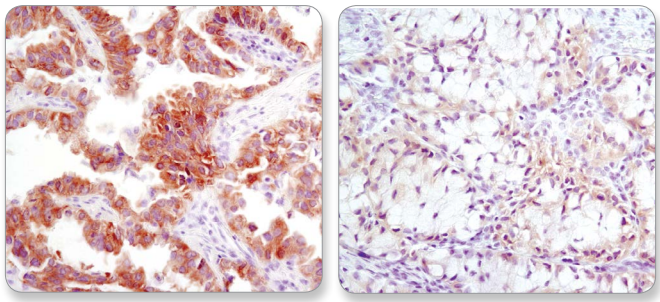
Carcinoma-associated fusion proteins discovered as a result of PTMScan® Technology

Further investigation revealed fusion of the N-terminus of EML4 (echinoderm microtubule-associated protein-like 4) with the C-terminus of ALK in some NSCLC cell lines and tumors. Additional studies showed that 3-7% of NSCLC patients express the fusion protein in their tumors, suggesting that it is highly oncogenic **(1-4)**. Cancer cells expressing the EML4-ALK fusion protein are sensitive to the small molecule ALK inhibitor crizotinib and in 2011 the FDA approved crizotinib for the treatment of ALK positive NSCLC **(5)**.



ALK fusion protein detection in human lung carcinoma

CST developed a highly specific and sensitive antibody, ALK (D5F3®) XP® Rabbit mAb #3633, which detects full length ALK and the EML4-ALK fusion protein. The FDA approved an immunohistochemistry (IHC) companion diagnostic test that uses the ALK D5F3 clone licensed from CST **(6)**. This will help physicians determine which NSCLC patients are candidates for crizotinib treatment.



ALK (D5F3®) XP® Rabbit mAb #3633: IHC analysis of paraffin-embedded human lung carcinoma with high (left) and low levels (right) of ALK fusion protein expression using #3633.

References:

1. Rikova, K. et al (2007) *Cell* 131(6), 1190–203.
2. Soda, M., et al (2007) *Nature* 448(7153), 561–6.
3. Koivunen, J.P., et al (2008) *Clin. Cancer Res.*14(13), 4275–83.
4. Shaw, A.T., et al (2009) *J. Clin. Oncol.* 27(26), 4247–53.
5. Press Release: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm269856.htm
6. Press Release: http://www.ventana.com/site/page?view=press-release-jan21-2015

Support for Drug Discovery

Integrated Services and Tools

In addition to proteomic services and kits, CST offers products for a suite of integrated applications to support different stages of the drug discovery and development pipeline, and to enable diagnostic development.

CST Services and Tools	Target Identification Validation	Hit/Lead Discovery	Efficacy/Tox Biomarkers
Proteomic Analysis » Post-Translational Modifications Analysis » Complete Service & Kit Portfolios	●	●	●
Immunofluorescence (ICC, IHC) » Cellular/Tissue Biology Systems Analysis » Single/Multiplex Marker Analysis	●	●	●
Flow Cytometry » Cellular, Single/Multiplex Analysis	●	●	●
ELISA Assays » Low to High Throughput Screen	●	●	●
Antibody Arrays » Fast to Medium Throughput Profiling	●	●	●
ChIP Analysis/Sequencing » Gene Analysis	●	●	
siRNA Portfolio » Gene Analysis	●		
Product Customization » Development/Labeling/Formulation/Scale Production	●	●	●

Troy, Product Scientist, has been with CST since 2010.

Technical Support

At CST, providing exceptional customer service and technical support are top priorities. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

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