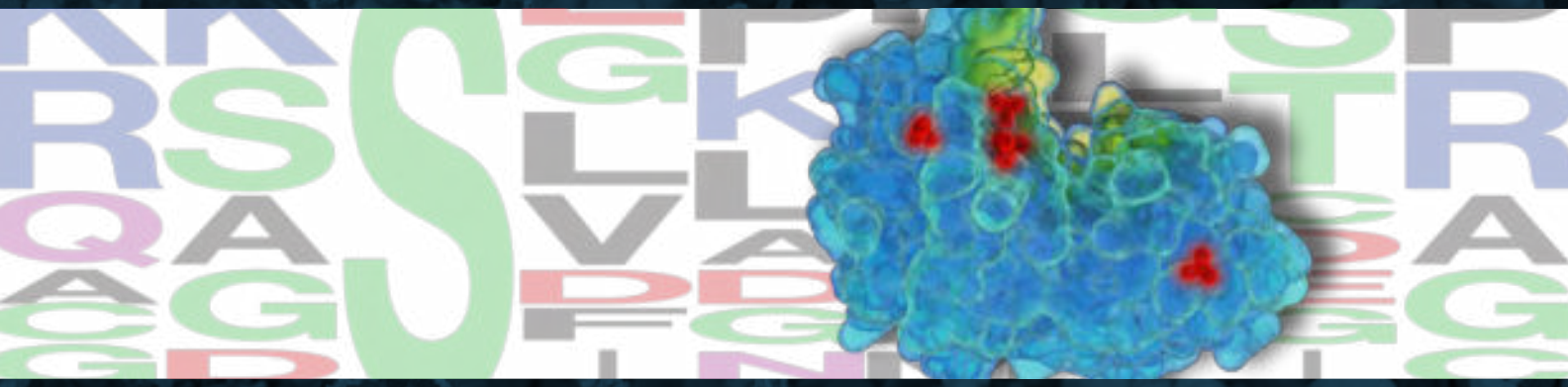


Simplifying Proteomics

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

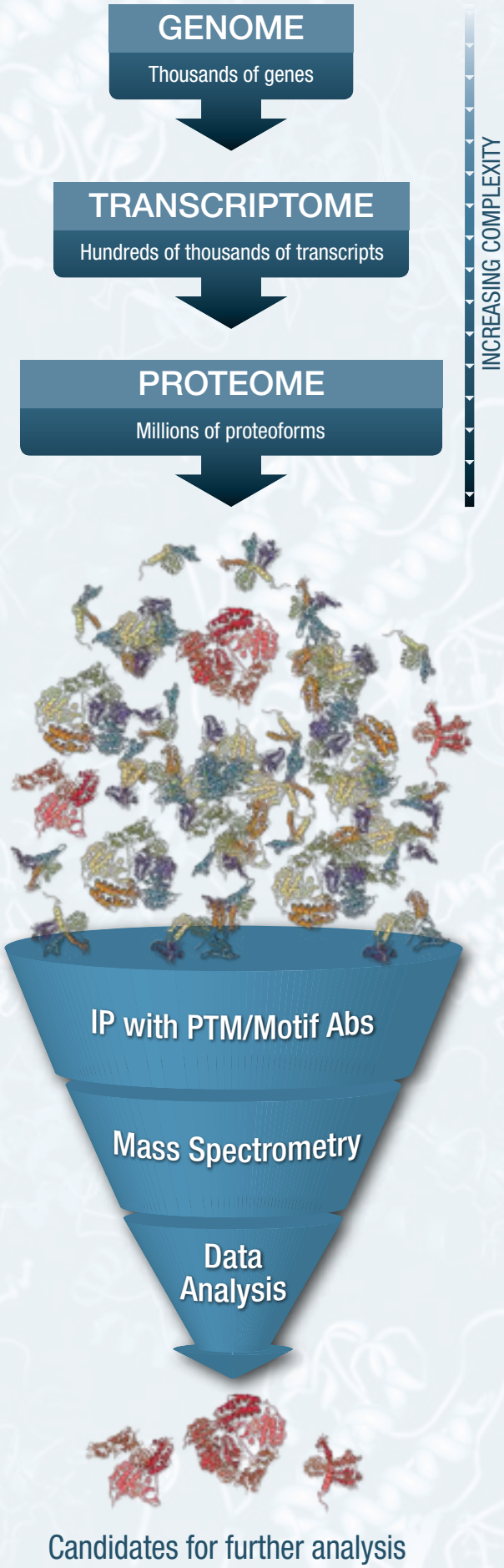
PTMScan®: Antibody enrichment of modified peptides for mass spectrometry-based proteomics

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

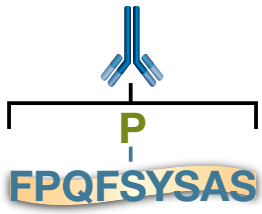
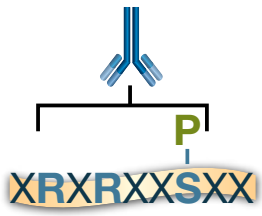
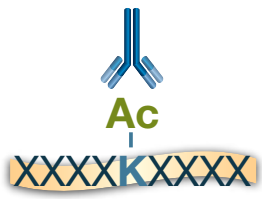


Key to Success: PTMScan® Antibodies

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

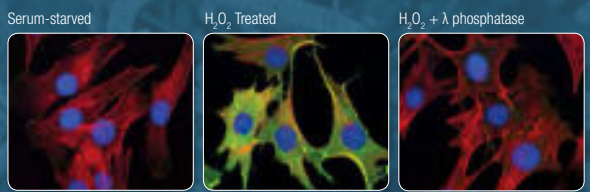
The table below outlines the three types of antibodies for PTMScan technology.

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.

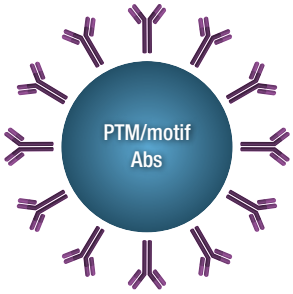
For full list of MultiMab antibody mixtures visit:
www.cellsignal.com/MultiMab



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.§

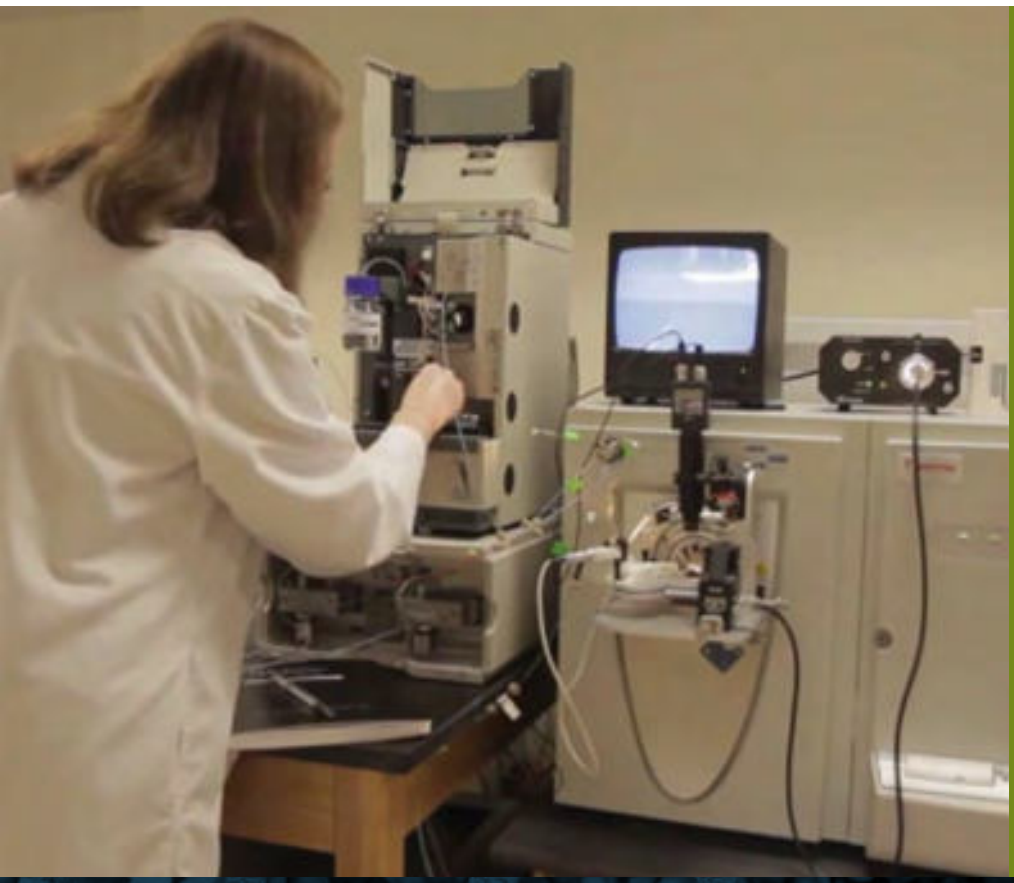
*A smaller format for certain kits (3 assays instead of 10) allows investigators to run pilot studies.

§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

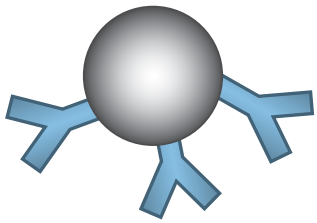


PTMScan® Discovery and Direct Services

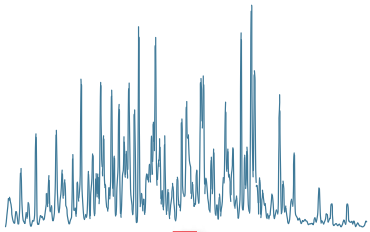
Workflow

Protease-digested Sample

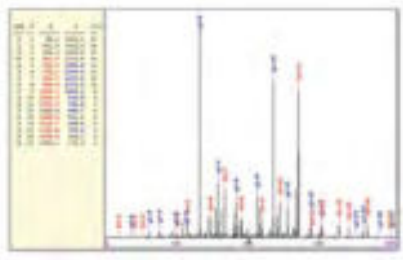
PTMScan® Reagent Immobilized to Protein-A or -G Agarose



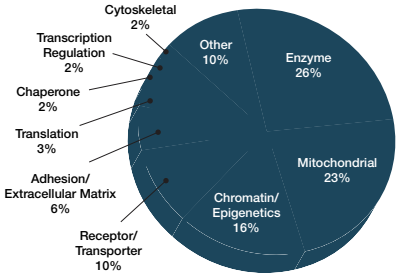
Analyze Eluted Peptide Fraction by LC-MS/MS

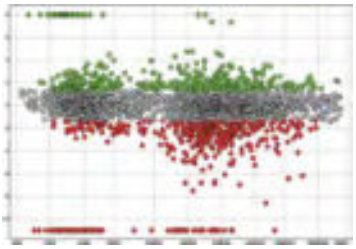


Assign Sequences to MS/MS Spectra with SEQUEST®



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*FAEPGGGGGGGGGGPGGSASGPGGTGGGK	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.