

# PTMScan<sup>®</sup> Discovery

## Proteomics Services and Kits

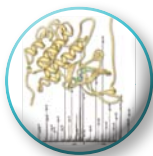


Cell Signaling

TECHNOLOGY<sup>®</sup>

# PTMScan<sup>®</sup> Proteomics Services

PTMScan<sup>®</sup> Proteomics Services from Cell Signaling Technology (CST) employ proprietary methodologies for antibody-based peptide enrichment combined with tandem mass spectrometry for quantitative profiling of post-translational modifications (PTMs), including phosphorylation, ubiquitination, and acetylation.



## PTMScan<sup>®</sup> Discovery

A proteomics method for the identification and quantitation of known and novel post-translational modifications (PTMs), including Ser, Thr, and Tyr phosphorylation, as well as ubiquitination and acetylation. The method employs immunoaffinity purification using CST's proprietary motif antibodies coupled with tandem mass spectroscopy for comprehensive profiling of up to thousands of PTMs from cell line and tissue samples. This discovery-mode proteomics technology is useful for the study of PTMs throughout the proteome in a variety of biological model systems and disease states.



## PTMScan<sup>®</sup> Direct

A multiplex proteomics assay for quantitative measurement of a defined set of known PTMs on critical signaling nodes within a group of cellular signaling pathways. The method employs an immunoaffinity purification technique using CST's proprietary modification state-specific antibodies coupled with mass spectrometry, allowing for targeted screening of hundreds of defined signaling proteins from cell line and tissue samples. This targeted-mode proteomics technology is used to investigate changes in PTMs to specific or known protein targets in response to a drug treatment or disease state.

PTMScan<sup>®</sup> Inquiries

[www.cellsignal.com/PTMSinquiries](http://www.cellsignal.com/PTMSinquiries)

# Applications

## Target Validation

PTMScan® Proteomics Services have been used most extensively to discover novel mechanisms of disease based on changes in PTM profiles associated with aberrantly activated kinases in disease models. PTMScan® Technology is equally effective in the identification of drug targets and potential off-target effects, mechanism-of-action studies, and the elucidation of cellular regulatory networks.

## Pathway Profiling

PTMScan® Proteomics Services allow monitoring of the activation of up to thousands of cellular signaling proteins across a broad range of pathways.

## Biomarker Discovery

PTMScan® Proteomics Services provide a strategy for profiling lead compounds and performing proof-of-mechanism and proof-of-concept studies. This enables the discovery and validation of biomarkers that predict compound activity, disease susceptibility, and drug response.

## Patient Stratification

PTMScan® Proteomics Services enable correlation of target enzyme activation and drug sensitivity, which can provide the basis for patient stratification assay development.

# Deliverables

## Summary Report

The report contains qualitative and quantitative results, analysis, interpretation, and recommendations.

The following is provided in table format with detailed explanation of contents and guidelines for data review:

- Peptide Sequences
- Quantitative changes at sites of PTMs using label-free or SILAC methods
- Parent proteins and functions
- Classification information
- DTA/mzXML ion spectra for client internal analysis

## Consultation

Consultation with CST scientists for discussion and review of the results.



# PTMScan® Discovery Proteomics Services

## Advanced Proteomic Technology for Target and Biomarker Discovery

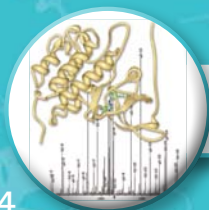
PTMScan® Proteomics Technology, developed at Cell Signaling Technology (CST), provides comprehensive analytical profiling of post-translational modifications (PTMs), including serine/threonine and tyrosine phosphorylation (PhosphoScan® Technology), ubiquitination (UbiScan® Technology), and acetylation (AcetylScan® Technology).

PTMScan Technology employs CST™ Motif Antibodies for peptide immunoaffinity purification from protease-digested cell extracts combined with LC tandem mass spectrometry (LC-MS/MS) to identify and quantify changes in modification state levels. Motif Antibodies are specific to Ser/Thr/Tyr-phosphorylated peptides, acetylated peptides, or ubiquitin-tagged peptides, enabling the analysis of phosphorylation, acetylation, or ubiquitination status of the cell.

PTMScan Services, performed by CST scientists, deliver hundreds to thousands of protein PTM identifications and quantification of PTM changes in customer samples. PTMScan Services include proprietary sample fractionation, phosphopeptide immunoaffinity purification with our optimized PTMScan Motif Antibodies, and identification using LC-MS/MS. This is followed by extensive informatic data analysis and a consultation between in-house PTMScan Technology experts and the customer.

## PTMScan® Discovery Service

Includes a qualitative and quantitative results table, an informatics results table, a summary presentation, and a copy of the raw LC-MS data files in mzXML format.

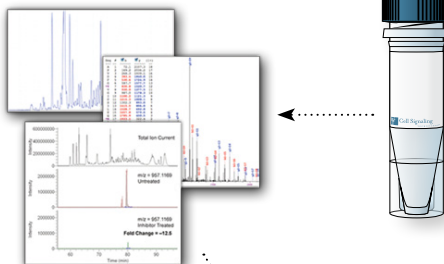
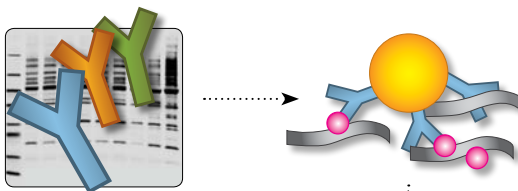


**PTMScan® Inquiries:** Visit: [www.cellsignal.com/PTMSinquiries](http://www.cellsignal.com/PTMSinquiries)

# Workflow

## Step 1: Analysis

- Cell line and/or tissue samples ± treatment; Protease digestion.
- Quality assurance of samples by western blot.
- Peptide immunoaffinity purification (IAP).
- Tandem mass spectrometry (LC-MS/MS) analysis of enriched peptides for qualitative sequence and modification site identification.
- Quantitative analysis of peptide fold-change between study samples.

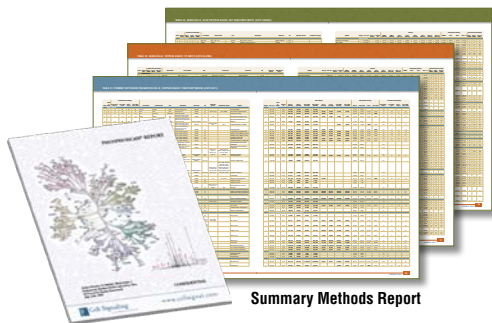


## Step 2: Report and Consultation

- Report with qualitative and quantitative results.
- Report contains sequence and corresponding protein identification in table format, detailed explanation of table contents, and guidelines for data review.
- Detailed discussion and review of report with PTMScan Technology experts.
- Typical timeline: approximately 3 to 4 weeks; timeline will vary with project size.

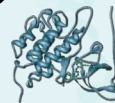


Summary Presentation



Summary Methods Report

# PTMScan® Discovery Proteomics Services



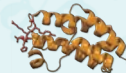
## PhosphoScan® (Phosphorylation Proteomics)

**Tyrosine PhosphoScan® Services:** Tyrosine PhosphoScan® Services provide a comprehensive strategy for global phosphotyrosine profiling. The Cell Signaling Technology (CST) P-Tyr-1000 mAb is more broadly reactive with phosphotyrosine peptides than any other antibody. Employing P-Tyr-1000 for phosphopeptide enrichment in combination with LC-MS/MS analysis yields quantitative profiles of thousands of non-redundant phosphorylated sequences.

Tyrosine PhosphoScan Proteomics is used routinely and successfully for target discovery, mutant RTK screening, lead compound characterization, and biomarker discovery.

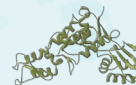
**Serine/Threonine PhosphoScan® Proteomics:** KinomeView® and PhosphoScan Proteomics Services together are a two-step process enabling kinome-wide quantitative analysis of cellular phosphorylation for the discovery and validation of signaling targets, kinase substrates, and response biomarkers. KinomeView Service is a western blot-based assay that provides a first step to guide the design of PhosphoScan studies by determining which branches of the kinome are responsive to the treatment condition under investigation. KinomeView can identify which Phospho-Motif Antibodies will yield the greatest insights when used in PhosphoScan mass spectrometry analysis. PhosphoScan Services provide in-depth, yet focused phosphoproteomic analysis with patented CST™ Phospho-Motif Antibodies. Each of these antibodies react broadly with diverse sets of serine-, threonine- and tyrosine-phosphorylated sequences.

## AcetylScan® (Acetylation Proteomics)



AcetylScan® Services are a unique and unparalleled strategy for global analysis of HDAC and HAT activity on protein acetylation. CST employs antibodies optimized for high affinity to acetylated-lysine (Ac-K) peptides. Our AcetylScan Services utilize these antibodies for enrichment of acetylated peptides from protease digested cell samples, followed by LC-MS/MS analysis for quantitative profiles of thousands of non-redundant acetylated sequences.

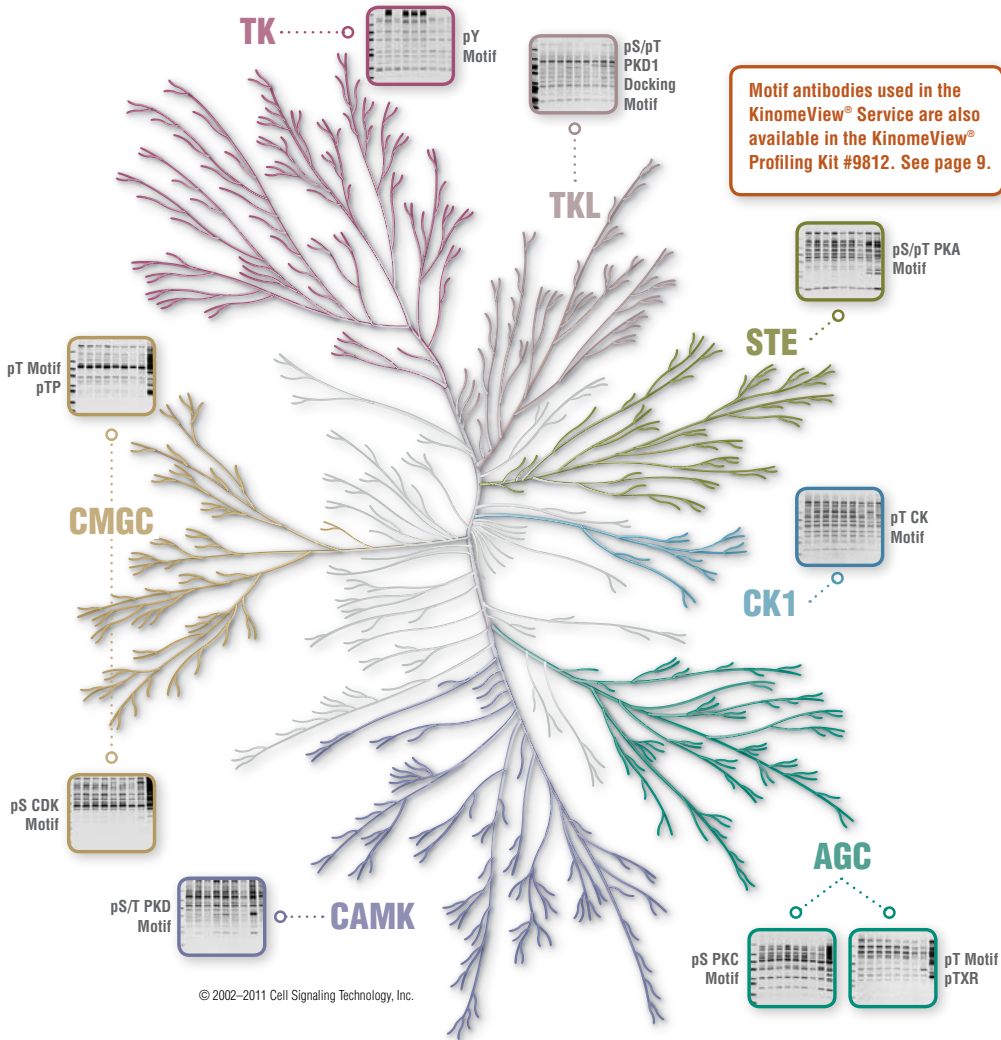
## UbiScan® (Ubiquitination Proteomics)



Ubiquitination is an important mechanism for both signal transduction and proteolytic degradation. The UbiScan® Proteomics method is a unique and unparalleled strategy for global analysis of cellular ubiquitination. CST has developed a proprietary ubiquitin branch (“K-GG”) XP® monoclonal antibody with specificity for a di-glycine remnant on ubiquitinated lysine residues after trypsin digestion. In UbiScan Services, the K-GG antibody is employed on trypsin digested samples to enrich ubiquitinated peptides, followed by LC-MS/MS analysis for quantitative profiles of thousands of non-redundant ubiquitinated sequences. UbiScan Proteomic Services are applied in comparative studies to identify substrates and biomarkers of ubiquitin pathway targets and compounds.

# KinomeView® Profiling Service

The serine/threonine phosphoproteome is extremely complex. KinomeView® Service is a western blotting service that employs CST™ Motif Antibodies with broad reactivity for serine, threonine, and tyrosine phosphorylation to provide a kinome-wide view of cellular phosphorylation. Before beginning a PhosphoScan Service, the objective of KinomeView Service is to identify which Phospho-Motif Antibodies detect phosphorylation responsive to the treatment under investigation. The Motif Antibodies that reveal treatment-mediated changes by KinomeView western blotting will be the same antibodies used in subsequent PhosphoScan Services for LC-MS/MS analysis.



Additional CST™ Motif Antibodies are available for KinomeView Service.

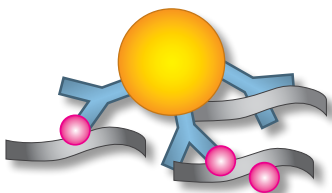
# PTMScan® Proteomics Product Offering

A collection of products enable you to access and use Cell Signaling Technology's patented PTMScan® Technology, as a complement to PTMScan Services.

## PTMScan® Discovery Motif Antibody Kits

PTMScan® Motif Antibody Kits are a product line that enables the use of PTMScan Technology, a proprietary technology developed at CST. The kits provide a selection of our proprietary Motif Antibodies ready for use in studies that employ PTMScan Technology. These antibodies are identical to those utilized by CST scientists in PTMScan Discovery Services. For our customers with in-house expertise in mass spectrometry proteomics, these kits provide a cost-effective means of accessing a selection of CST™ Motif IAP Beads and a Limited Use License to perform PTMScan Technology.

Reagents for 10 assays as well as detailed protocols are provided in each kit.



Target	Kit	Consensus Site
Acetylated-Lysine	#13416	Ac-K
Phospho-Akt Substrate	#5561	(RXXS*/T*)
Phospho-Akt Substrate	#5563	RXRXX(S*/T*)
Phospho-(Ser/Thr) AMPK Substrate	#5564	(L/M)XRXX(S*/T*), RXX(S*/T*)
Phospho-(Ser) ATM/ATR Substrate	#12267	S*Q
Mono-Methyl Arginine	#12235	Me-R
Asymmetric Di-Methyl Arginine	#13474	Me-R
Symmetric Di-Methyl Arginine	#13563	Me-R
Cleaved Caspase Substrate	#12810	DE(T/S/A/D)
Phospho-(Ser/Thr) CK2 Substrate	#12170	(S*/T*)(D/E)X(D/E)
Phospho-MAPK/CDK Substrates	#4652	PXS*P, S*PX(K/R)
Phospho-PKA Substrate	#5565	(K/R)(K/R)X(S*/T*)
Phospho-Tyrosine (P-Tyr-100)	#5636	Y*
Phospho-Tyrosine (P-Tyr-1000)	#8803	Y*
Phospho-Thr-Pro Motif	#5567	T*P, T*PP
Phospho-(Thr) PLK Binding Motif	#5566	ST*P
Succinyl-Lysine	#13764	Succ-K
Ubiquitin Branch	#5562	K-ε-GG

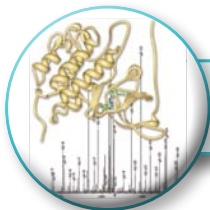


## KinomeView® Profiling Kit #9812

Provides a set of Phospho-Motif Antibodies that covers a large portion of the kinome and reacts broadly with serine, threonine, and tyrosine phosphorylation mediated by diverse kinase families. This kit provides researchers with a western blotting strategy to dissect the complexity of the phosphoproteome and determine the kinase families involved in the regulation of diverse physiological processes.

Products Included	Motif	Isotype
#9614 Phospho-Akt Substrate (110B7E) Rabbit mAb	(RXXS*/T*)	Rabbit IgG
#10001 Phospho-Akt Substrate (23C8D2) Rabbit mAb	RXRX(S*/T*)	Rabbit IgG
#5759 Phospho-(Ser/Thr) AMPK Substrate Rabbit mAb	(L/M)XRX(S*/T*), RXX(S*/T*)	Rabbit IgG
#9607 Phospho-(Ser) ATM/ATR Substrate Rabbit mAb	S*Q	Rabbit IgG
#6966 Phospho-(Ser/Thr) ATM/ATR Substrate Rabbit mAb	(S*/T*)QG, (S*/T*)Q	Rabbit IgG
#9477 Phospho-(Ser) CDK Substrate Antibody	(K/H)S*P	Rabbit IgG
#8738 Phospho-(Ser/Thr) CK2 Substrate Antibody	(S*/T*)(D/E)X(D/E)	Rabbit IgG
#2325 Phospho-MAPK/CDK Substrates (34B2) Rabbit mAb	PXS*P, S*PX(K/R)	Rabbit IgG
#9634 Phospho-(Ser/Thr) PDK1 Docking Motif (18A2) Mouse mAb	(F/Y)(S*/T*)(F/Y)	Mouse IgG2a
#9624 Phospho-PKA Substrate (100G7E) Rabbit mAb	(K/R)(K/R)X(S*/T*)	Rabbit IgG
#6967 Phospho-(Ser) PKC Substrate Rabbit mAb	(K/R)XS*X(K/R)	Rabbit IgG
#5243 Phospho-(Thr) PLK Binding Motif (D73F6H9) Rabbit mAb	ST*P	Rabbit IgG
#3003 Phospho-Thr-Pro Motif Antibody	T*P, T*PP	Rabbit IgG
#3004 Phospho-Thr-Pro-Glu Motif (C32G12) Rabbit mAb*	T*PE, T*P	Rabbit IgG
#2351 Phospho-Thr-X-Arg Motif Antibody	T*XR, T*PR	Rabbit IgG
#8954 Phospho-Tyrosine Rabbit mAb (P-Tyr-1000)	Y*	Rabbit IgG
#7074 Anti-rabbit IgG, HRP-linked Antibody	–	Goat
#7076 Anti-mouse IgG, HRP-linked Antibody	–	Horse

\* Select antibodies contained in the KinomeView Profiling Kit are not available for individual sale.



**PTMScan® Inquiries:** Visit: [www.cellsignal.com/PTMSinquiries](http://www.cellsignal.com/PTMSinquiries)

# PTMScan® Proteomics System

Cell Signaling Technology (CST) offers a Per-Use License with the PTMScan® Proteomics System to provide our customers with in-house expertise in mass spectrometry proteomics with a cost-effective means of accessing CST™ Motif IAP Beads and proprietary methodology. This system provides any of our Motif IAP Beads validated and ready for use in PhosphoScan®, UbiScan®, AcetylScan®, MethylScan®, SuccinylScan®, or cleaved caspase substrate experiments.

## Flexibility

System is built-to-order; the Motif Antibodies included and the amount of each can be varied according to your specification and the number of analyses you intend to perform.

## Consistent Performance

Each bead-coupled Motif Antibody in the system is custom prepared and validated prior to shipment to ensure consistent and high quality performance.

## Support

Scientists with extensive PTMScan Technology experience are available to provide technical support. This system also includes a detailed protocol describing cell sample preparation, IAP enrichment, and peptide sample preparation for LC-MS/MS analysis.

## System Components

- Motif IAP Beads – *One or more available*
- IAP Buffer
- PTMScan® Protocol
- PTMScan® Per-Use License



**NOTE:** The PTMScan® Proteomics System provides a limited Per-Use License that is restricted to allow performance of CST™ PTMScan methods with only Motif IAP Beads purchased as part of the CST PTMScan Proteomics System.

## Products for Follow-up Validation Studies

Several reagents from Cell Signaling Technology's product offering are readily available for follow-up target validation studies after completion of the PTMScan® Service Project. A selection of these products is highlighted and hyperlinked from the PTMScan Service Results Summary Reports for our clients' convenience.

# AQUA™ Peptide Synthesis

To validate and quantify novel findings through PTMScan Technology, CST offers custom-synthesized, stable isotope-labeled AQUA™ peptides. AQUA peptides enable isotope dilution strategies, which are the gold standard of quantitative mass spectrometry. We specialize in high-quality custom AQUA peptide synthesis, including challenging sequences and unusual post-translational modifications. We have hundreds of synthetic AQUA peptides in stock, and turn-around on new AQUA peptide synthesis and quality control is approximately 4–6 weeks.

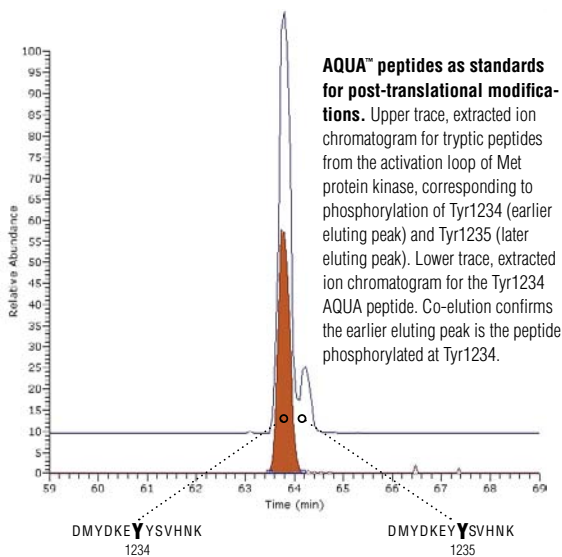
This method was developed by scientists at Harvard Medical School. Limited use of this method is permitted under a licensing arrangement with Harvard College.

- ⚡ Peptides are typically ~20 residues or less in length but longer lengths are available
- ⚡ Modification options include: phosphorylation; acetylation; ubiquitination; cysteine alkylation; methyl-arginine, methyl-histidine, and methyl-lysine
- ⚡ Heavy residues: leucine, valine, proline, phenylalanine, alanine, and glycine can be incorporated
- ⚡ No minimum order

## AQUA™ Peptide Inquiries:

Find order information online at:

[www.cellsignal.com/orderinfo](http://www.cellsignal.com/orderinfo)



- ⚡ **Modification state-specific and total protein antibodies, including our exclusive line of XP® monoclonal antibodies,** are produced and validated in-house in a number of applications, including western blotting, immunohistochemistry, and immunofluorescence.
- ⚡ **SignalSilence® siRNA duplexes** that allow knockdown of specific human or mouse proteins are available for many targets.
- ⚡ **PathScan® Sandwich ELISA Kits and Antibody Pairs** are available to measure a large selection of intracellular signaling molecules. These products are developed, produced, and validated in-house, ensuring robust, sensitive, and specific assays.



Hongying, Research Associate,  
has been with CST since 2006.

## FOUNDED BY RESEARCH SCIENTISTS IN 1999,

Cell Signaling Technology (CST) is a private, family-owned company with over 400 employees worldwide. Active in the field of applied systems biology research, particularly as it relates to cancer, CST understands the importance of using antibodies with high levels of specificity and lot-to-lot consistency. It's why we produce all of our antibodies in house, and perform painstaking validations for multiple applications. And the same CST scientists who produce our antibodies also provide technical support for customers, helping them design experiments, troubleshoot, and achieve reliable results. We do this because that's what we'd want if we were in the lab. Because, actually, we are.

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