PTMScan® Direct
Proteomics Services

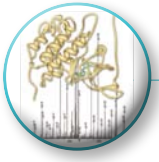
Cell Signaling TECHNOLOGY®

PTMScan® Proteomics Services

PTMScan® 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® 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® 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® Inquiries

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.

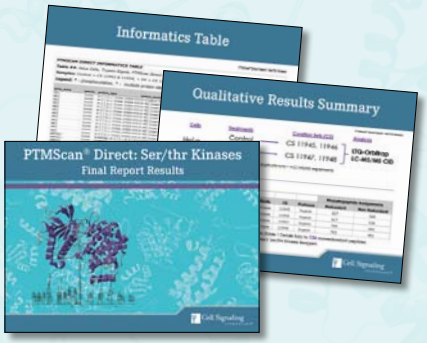
Deliverables

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® Direct Proteomics Services

PTMScan® Direct, a proprietary technology developed by Cell Signaling Technology (CST) scientists, provides targeted screening and quantification of a defined set of protein sites and signaling nodes critical for drug discovery, biomarker discovery, or diagnostic development. PTMScan® Direct employs an immunoaffinity purification method using proprietary modification site-specific antibodies coupled with LC-MS/MS. The method enables a multiplex proteomics assay for quantitative measurement of a defined set of critical signaling nodes within a group of cellular signaling pathways, and can be followed up with traditional antibody screening methods. The highest quality antibody products employed in PTMScan® Direct Proteomics Services are readily available from CST for many of the endpoint markers (see pg 11).

Features and Benefits

- Identification and quantification of up to hundreds of signaling nodes from multiple signaling pathways, enabling the most comprehensive pathway analysis profiling available.
- Direct measurement of specific peptide targets using LC-MS/MS alleviates background levels that are intrinsic to other protein-based assays, and allows for greater assay sensitivity and enhanced dynamic range.
- The start-to-finish service, performed by CST scientists with over 30 years of combined LC-MS expertise, provides you with timely and high-quality results.
- Deliverables include comprehensive result tables and a Summary Report containing a study overview and a results table guide, facilitating independent analysis and dissemination of your results.

PTMScan® Direct Service

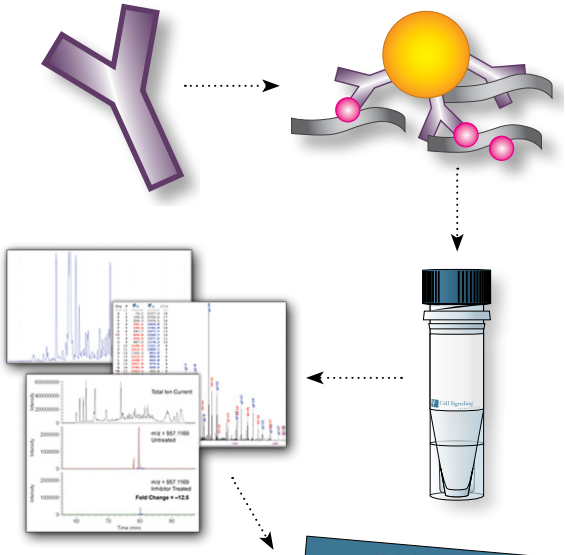
Includes a qualitative and quantitative results table, an informatics results table, a summary presentation, and a copy of the raw LC-MS data files in mzXML format. The Standard PTMScan® Direct data package will be provided to the customer in electronic form using a secure ftp link.

PTMScan® Inquiries: Visit: **www.cellsignal.com/PTMSinquiries**

Workflow and Deliverables

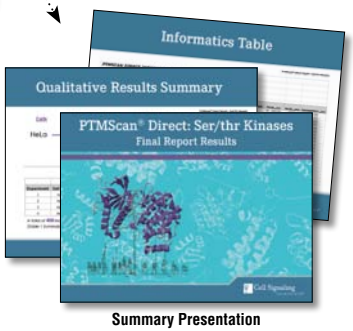
Step 1: Analysis

- Experimental objectives and design consultation with CST scientists.
- Determine cell line or tissue samples and experimental parameters for study.**
- Peptide immunoaffinity purification (IAP) with proprietary antibody reagent.
- Tandem mass spectrometry (LC-MS/MS) analysis of enriched phosphopeptides for qualitative sequence and phosphorylation site identification.
- Quantitative analysis of phosphopeptide fold-change between study samples for the endpoints defined in the service.

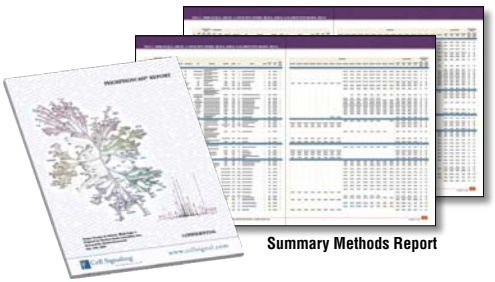


Step 2: Report and Consultation

- PTMScan® Direct report with qualitative and quantitative results.
- Report contains sequence assignments in table format and detailed explanation of table contents and guidelines for data review.
- Detailed discussion and review of report with CST scientists.
- Typical timeline: approximately 5 weeks; preliminary results delivery in 2 to 3 weeks; timeline will vary with project size.



Summary Presentation



Summary Methods Report

* Limited customization: During initial consultation, customized inclusion of a limited number of additional protein sites into the assay can be accommodated. Contact **ptmscan@cellsignal.com** for more details.

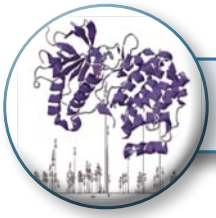
* β-actin/Histone H3, GAPDH, and α-Tubulin are included as total protein control markers.

PTMScan® Direct–Ser/Thr Kinases

PTMScan® Direct Ser/Thr Kinases Service is a proteomics method for quantitative measurement of the activation state of a defined set of Ser or Thr kinases. The method employs immunoaffinity purification using CST's proprietary modification site-specific antibodies directed against known Ser, Thr, or Tyr sites on kinases throughout the Ser/Thr kinome, combined with tandem mass spectrometry for targeted screening of a defined set of Ser/Thr kinases. The PTMScan Direct Ser/Thr Kinases Service enables kinome-wide analysis of cellular phosphorylation for the validation of signaling targets, kinase substrates, and response biomarkers.

Ser/Thr phosphorylation events in various tissues using PTMScan® Direct Ser/Thr Kinases Reagent.
Mouse tissue hierarchical clustering. Intensities from the three mouse tissues (liver, brain, embryo) were normalized on a scale of 0 to 1 and clustered using euclidean distance in Spotfire DecisionSite® (V.9.1.2, TIBCO Software AB) **(A)**. Each row represents a different peptide ion, and the three columns represent the three tissue types. The more intense the **GREEN** shading for a particular cell the closer the measured signal is to the maximum intensity of 1.

Unclustered heat map of Akt and PKC peptides. The data in **(A)** was organized by kinase to examine peptide abundance for Akt family members **(B)** and PKC isoforms **(C)**.



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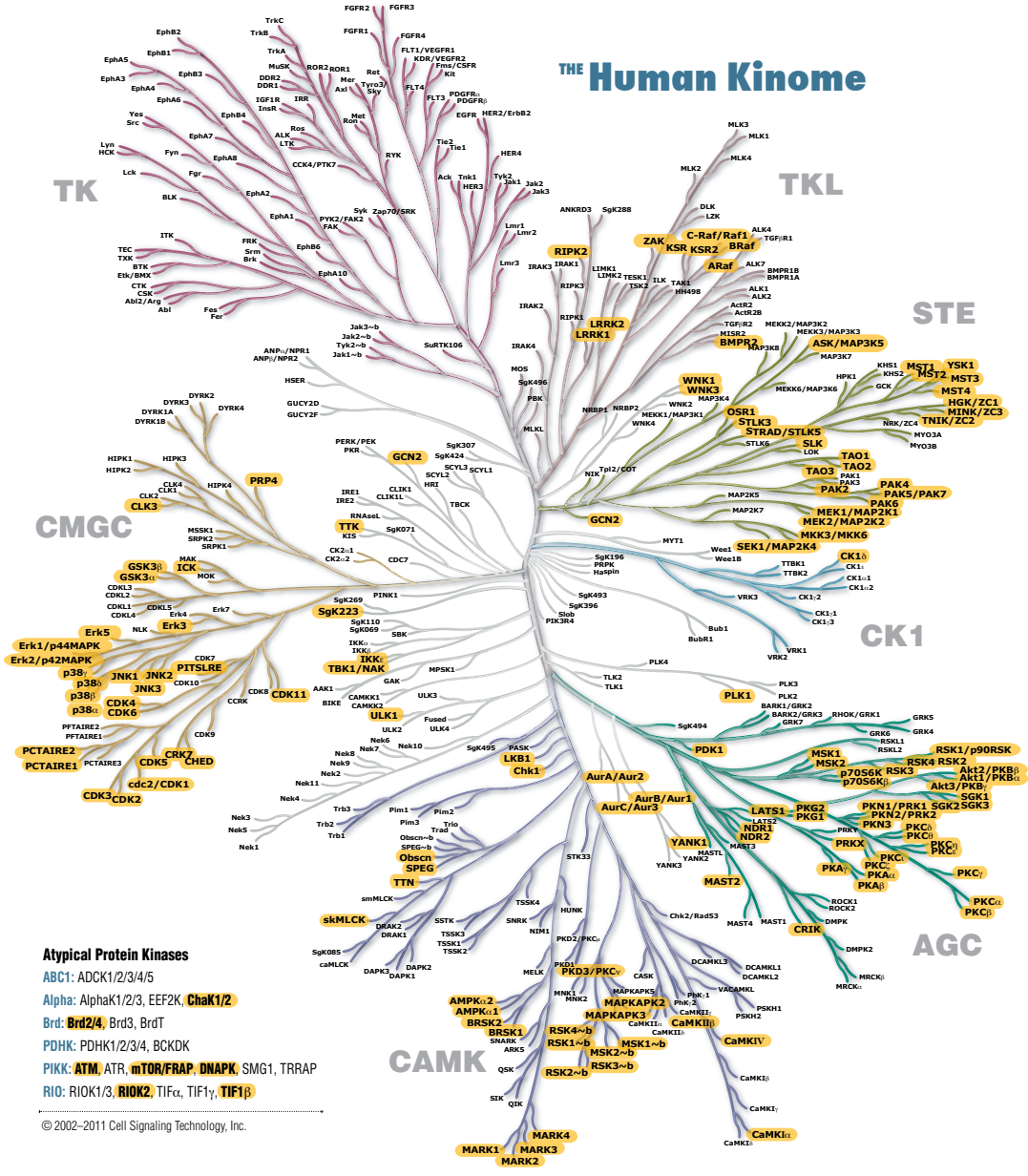
THE Human Kinome



Atypical Protein Kinases

ABC1: ADCK1/2/3/4/5

Alpha: AlphaK1/2/3, EEF2K, **ChaK1/2**

Brd: **Brd2/4**, Brd3, BrdT

PDHK: PDHK1/2/3/4, BCKDK

PIKK: **ATM**, ATR, **mTOR/FRAP**, **DNAPK**, SMG1, TRRAP

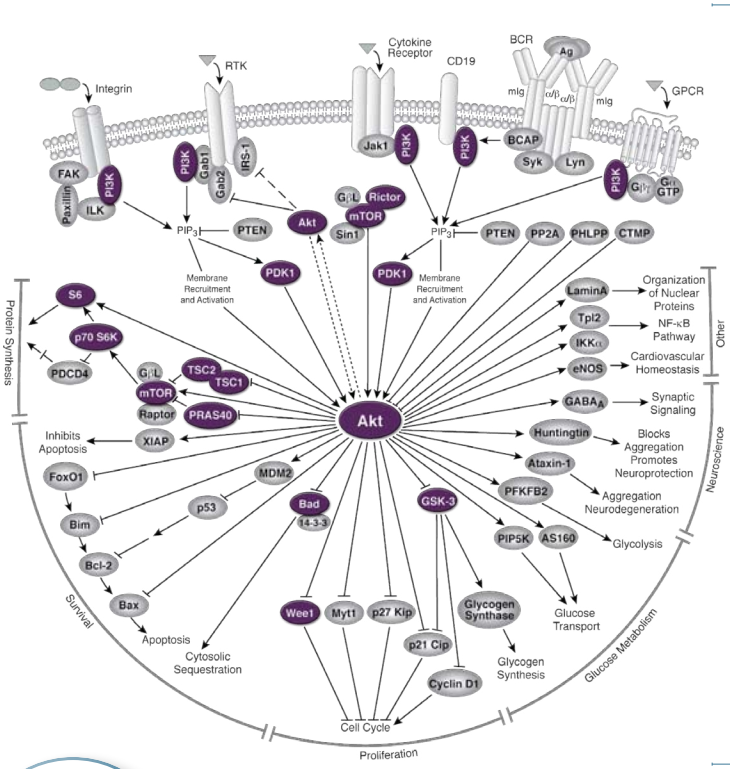
RIO: RIOK1/3, **RIOK2**, TIFα, TIF1γ, **TIF1β**

Highlighted proteins designate targeted protein modification sites within the human kinome that can be monitored using PTMScan® Direct Ser/Thr Kinases Service.

PTMScan® Direct–Multi-Pathway

PTMScan® Direct Multi-Pathway Service is a proteomics method for quantitative measurement of a defined set of phosphorylated proteins spanning a large number of signaling pathways, including Akt, MAPK, NF- B, and Jak/Stat signaling. The method employs an immunoaffinity purification method using CST's proprietary modification state-specific antibodies combined with tandem mass spectrometry for targeted screening of critical signaling nodes important for drug discovery, biomarker discovery, and diagnostic development.

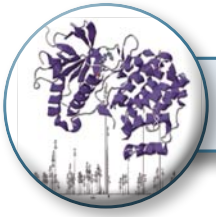
Highlighted purple nodes designate targeted protein modification sites within each sample signaling pathway that can be monitored with PTMScan® Direct Multi-Pathway Service.



Monitor phosphorylation in

PI3 Kinase/Akt Signaling

4EBP-2 (Thr37, Thr46) • Akt1 (Thr308, Thr312, Ser473, Thr479) • Akt2 (Thr309, Ser474, Ser478) • Bad (Ser75) • GSK-3 (Ser21) • GSK-3 (Ser9) • MDM2 (Ser166) • mTOR (Ser2448) • p70 S6 Kinase (Thr252, Thr412) • p70 S6 Kinase (Thr228) • PDK1 (Ser241) • PI3K p55 (Tyr199) • PI3K p85 (Tyr467) • PI3K p85 (Tyr464) • PRAS40 (Thr246) • Rictor (Ser1370, Ser1373, Thr1376) • S6 (Ser235, Ser236, Ser240, Ser244) • Wee1 (Ser642)



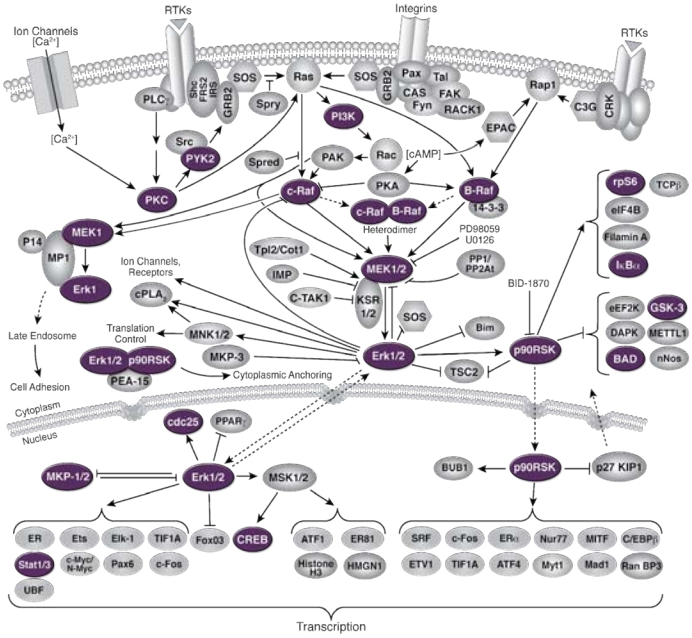
PTMScan® Inquiries: Visit: **www.cellsignal.com/PTMSinquiries**

Please visit www.cellsignal.com **for a complete list** of protein targets and phosphorylation sites monitored using this service.

Monitor phosphorylation in

MAP Kinase Signaling

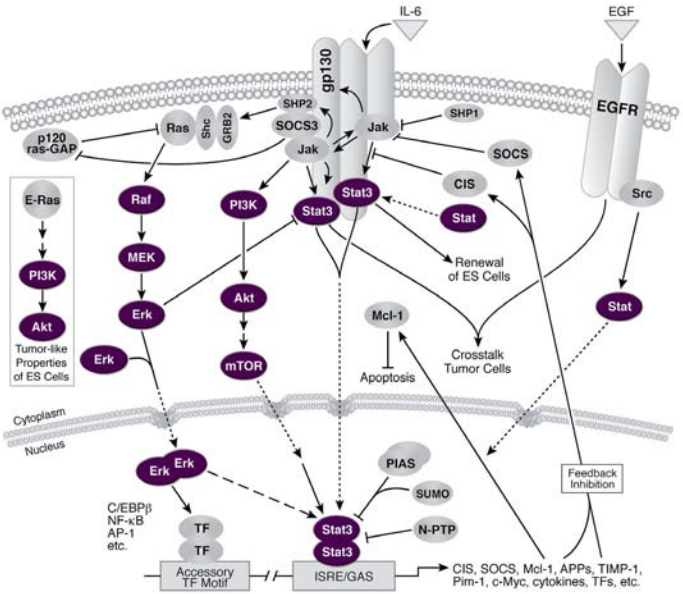
ATF-2 (Thr62, Thr69, Thr71) • Bad (Ser75) • B-Raf (Ser446) • c-Raf (Ser338) • cdc25C (Ser168, Ser216) • c-Jun (Ser73) • GSK-3a (Ser21) • GSK-3b (S9) • IkBa (Ser32, Ser36, Tyr42) • JNK3 (Thr221, Thr223) • JunD (Ser90, Ser100) • MEK1 (Ser217, Ser221) • MEK2 (Ser222, Ser226) • MKP-2 (Ser386) • p42 MAPK (Erk2) (Thr184, Tyr186) • p44 MAPK (Erk1) (Thr202, Tyr204) • p90RSK (Ser363, Thr359) • PI3K p85a (Tyr467) • PI3K p85b (Tyr464) • PKCa (Thr496) • Pyk2 (Tyr579, Tyr580) • RSK2 (Ser227) • RSK4 (Ser389) • S6 (Ser235, Ser236, Ser240, Ser244) • SAPK/JNK1 (Thr183, Tyr185) • Stat3 (Ser701, Tyr705, Ser727)



Monitor phosphorylation in

Jak/Stat Signaling

Akt1 (Thr308, Thr312, Ser473, Thr479) • Akt2 (Thr309, Ser474, Ser478) • B-Raf (Ser446) • c-Raf (Ser338) • MEK1 (Ser217, Ser221) • MEK2 (Ser222, Ser226) • mTOR (Ser2448) • p42 MAPK (Erk2) (Thr184, Tyr186) • p44 MAPK (Erk1) (Thr202, Tyr204) • PI3 Kinase p85 (Tyr467) • PI3 Kinase p85 (Tyr464) • Stat3 (Ser701, Tyr705, Ser727) • Stat5 (Tyr694) • Stat6 (Tyr641)



Additional Pathways Monitored with the Multi-Pathway Service: Apoptosis; Autophagy; Calcium, cAMP, and Lipid Signaling; Cell Cycle; Chromatin Regulation; Cytoskeletal Signaling/Adhesion; Metabolism; NF-kB Signaling; TGF-b Signaling; Translational Control; Wnt/b-Catenin Signaling.
Visit www.cellsignal.com for a complete list of protein targets and modification sites.

PTMScan® Direct–Multi-Pathway

The PTMScan® Direct Multi-Pathway Service can be used to investigate changes in the phosphorylation state on a defined set of signaling proteins in response to drug treatment or disease states. The data shown were generated from two representative experiments using the PTMScan Direct Multi-Pathway Service.

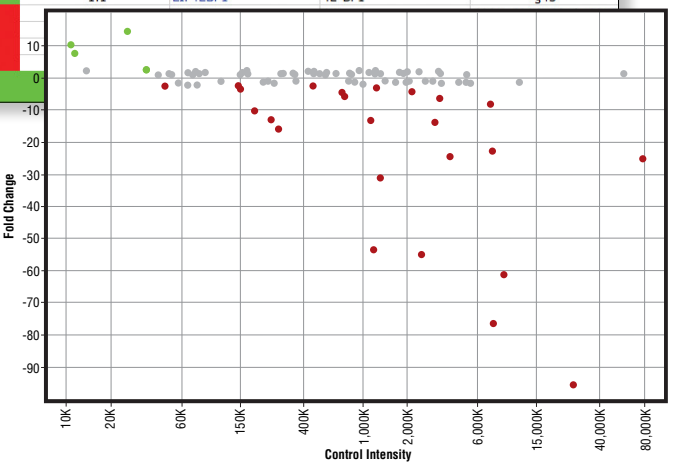
PTMSCAN® DIRECT RESULTS

Table #1: *H1703 Cells; Trypsin Digest; PTMScan® Direct Multi-Pathway*

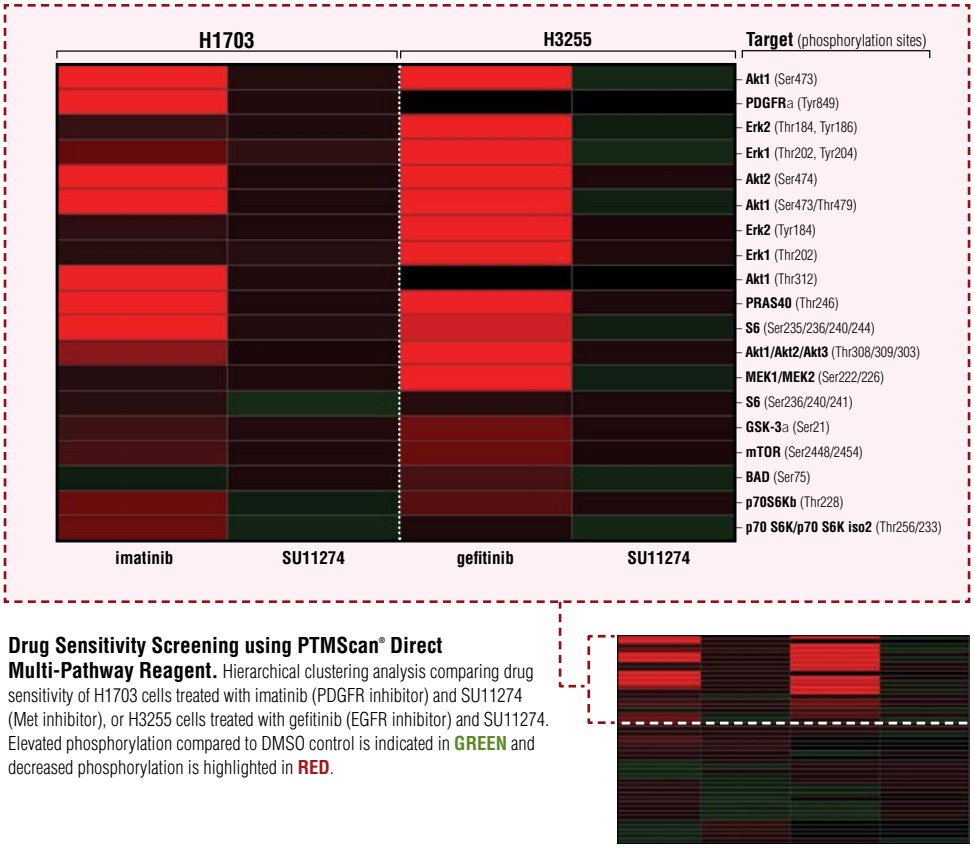
Samples: *imatinib = CS 11591; SU11274 = CS 11592; DMSO = CS 11593*

Legend: ******* *- phosphorylation,* ***#*** *- oxidized methionine,* ***§*** *- published site,* ***Blue Text*** *- CST antibody availa*

		Normalized Fold Change				
Index	**Index in Detail**	**Imatinib**	**SU11274 : DMSO**	**Gene Name**	**Protein Name**	**Site**
4	35	-15.9	-1.1	AKT1S1	PRAS40	§266
5	42	-3.2	1.0	BAD	BAD	§74
6	***Protein kinase, Ser/Thr (non-receptor)***					
7	56	-5.7	-1.0	AKT1; AKT2; AKT3	Akt1; Akt2; Akt3	305; 306; 302
8	57	-31.9	-1.2	AKT1	Akt1	§315
9	60	-105.6	-1.5	AKT1	Akt1	§473
10	61	-33.3	-1.1	AKT1	Akt1	§473, §479
11	64	-33.3	-1.1	AKT1	Akt1	§474, §479
12	66	-29.0	-1.1	AKT2	Akt2	§474
13	91	3.4	1.5	CDK2; CDK3	CDK2; CDK3	§14; §14
14	93	2.8	1.3	CDK2; CDK3	CDK2; CDK3	§15; §15
15	95	-2.6	-1.4	GSK3A	GSK3A	§19
16	96	-2.6	-1.4	GSK3A	GSK3A	§21
17	100	-2.4	-1.3	MAPK1	ERK2	§185, §187
18	107	-4.1	-2.0	MAPK3	ERK1	§202, §204
19	108	-2.5	-1.2	MAPK3	ERK1	§204
20	123	-3.0	-1.1	MTOR	mTOR	§2448, §2454
21	131	-3.4	-1.1	MTOR	mTOR	2450, §2454
22	151	-4.4	1.3	RPS6KB1; RPS6KB1	p70S6K; p70S6K iso2	248; 225
23	153	-4.4	1.3	RPS6KB1; RPS6KB1	p70S6K; p70S6K iso2	256; 233
24	154	-4.3	1.0	RPS6KB2	p70S6Kb	§228
25	***Protein kinase, Tyr (receptor)***					
26	155	-3.3	-1.5	PDGFRA	PDGFRa	§847
27	156	-108.9	-1.3	PDGFRA	PDGFRa	§849
28	***Transcriptional regulator***					
29	195	8.7	1.3	RB1	Rb	§778, §788
30	252	1.6	-2.6	SMAD1; SMAD9	Smad1; Smad8	§465; §467
31	253	1.3	12.4	SMAD5	Smad5	§462, §463
32	***Translation***					
33	362	2.6	-1.1	EIF4EBP1	4E-BP1	§41
34	399	2.6	-1.1	EIF4EBP1	4E-BP1	§44
35	418	2.6	-1.1	EIF4EBP1	4E-BP1	§45
36	444	-11.8				
37	445	-11.8				
38	446	-11.8				
39	448	-6.0				
40	452	19.3				
41	455	19.3				



Sample data from PTMScan® Direct Multi-Pathway study. Cells were lysed, and cellular proteins were digested with trypsin. The resulting peptides were subjected to immunoaffinity purification and LC tandem mass spectrometry using the PTMScan® Direct Multi-Pathway Reagent. Normalized fold changes of targeted phosphopeptides are shown, depicting positive **(GREEN)** and negative **(RED)** changes in modification state. Phosphopeptides exhibiting no change are shown in gray.



Drug Sensitivity Screening using PTMScan® Direct Multi-Pathway Reagent. Hierarchical clustering analysis comparing drug sensitivity of H1703 cells treated with imatinib (PDGFR inhibitor) and SU11274 (Met inhibitor), or H3255 cells treated with gefitinib (EGFR inhibitor) and SU11274. Elevated phosphorylation compared to DMSO control is indicated in **GREEN** and decreased phosphorylation is highlighted in **RED**.

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.

- **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.

By Region

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