

KinomeView™ to Serine/Threonine PhosphoScan® Proteomics

Step 1: KinomeView™ Study Design

KinomeView is western blot analysis with a set of 17 Phospho-Motif antibodies plus control antibodies to measure phosphorylation profiles in a kinome-centric manner and to guide PhosphoScan study design.

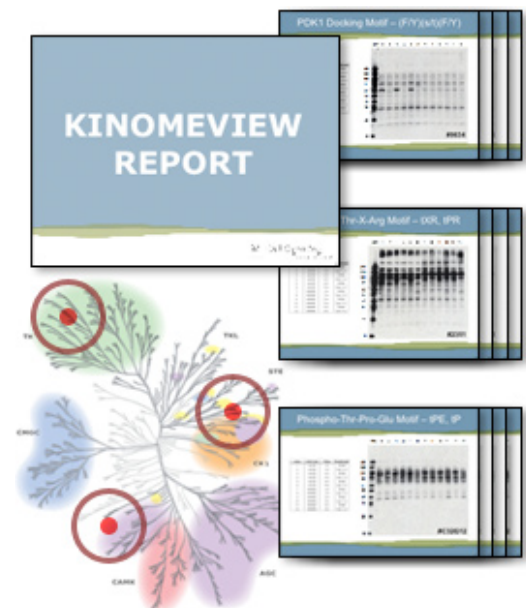
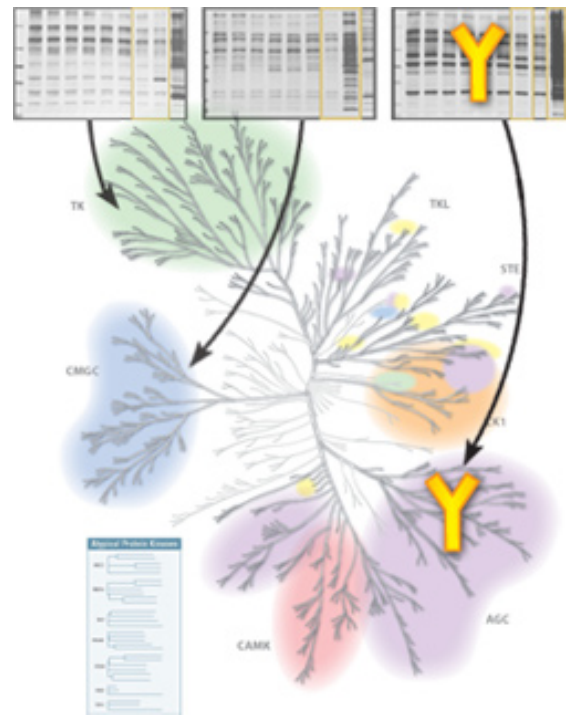
1. Study design begins with a teleconference or meeting with you to discuss how to apply KinomeView to the biology or chemistry under investigation.
2. KinomeView explores experimental parameters, including cell system, treatment doses and time points for PhosphoScan study design.
3. KinomeView control antibodies are included to measure experimental system-specific markers.

Step 2: KinomeView™ Analysis

1. For each of your sample sets, CST generates 19 western blots: one for each of 17 Phospho-Motif antibodies plus 2 positive control antibodies relevant to your experimental system.
2. KinomeView produces high-resolution western images.

Step 3: KinomeView™ Report and Consultation

1. KinomeView images are delivered as a report and as slides, along with specific recommendations for PhosphoScan studies and experimental parameters for analysis.
2. After you receive the report, CST scientists will review with you the KinomeView results, and discuss next-step PhosphoScan study design
3. KinomeView studies are completed in 2–4 weeks, and large comprehensive studies can be accommodated.



Cell Signaling

TECHNOLOGY®

PTMScan® Inquiries

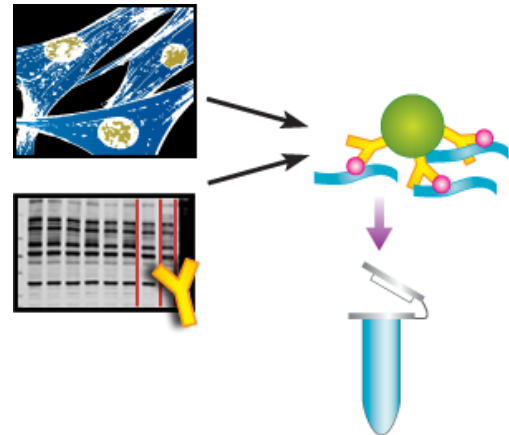
email: ptmscan@cellsignal.com

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Step 4: PhosphoScan® Proteomics Service Planning

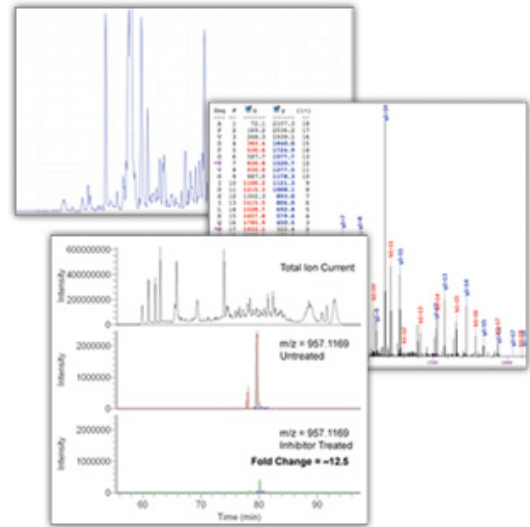
Next, the proteins and phosphorylation sites that showed pattern differences in KinomeView are identified by PhosphoScan, through immunoaffinity purification of phosphopeptides and mass spectrometry.

1. We will discuss with you the optimal PhosphoScan study design before you prepare samples.
2. We will also discuss with you and recommend quantification options to measure differences in phosphorylation resulting from sample treatments.
3. Planning includes coordinating our schedule for sample preparation and analysis with your project schedule and deadlines.



Step 5: PhosphoScan® Proteomics Analysis

1. Samples shipped to CST are validated by western blot with designated Phospho-Motif antibodies before starting PhosphoScan.
2. Samples are processed to protein extracts, which are digested with proteases and fractionated.
3. Whole proteome peptide samples are treated with the Phospho-Motif antibodies, chosen based on KinomeView, to enrich phosphorylation sites that caused pattern changes.
4. Enriched phosphopeptides and phosphorylation sites are identified by mass spectrometry (LC-MS/MS) analysis.
5. Treatment induced changes in phosphorylation levels are measured by either label-free or isotope-labeling (SILAC) quantification.



Step 6: PhosphoScan® Report and Consultation

1. PhosphoScan identification and quantification results are delivered as a report, which includes interpretation and recommendations by CST project scientists.
2. We provide a spreadsheet file listing peptide sequences and phosphorylation sites along with protein function and classification. We also provide detailed guidelines to help you mine the table.
3. After receiving the report, you will meet with CST scientists to review and discuss the PhosphoScan results.
4. PhosphoScan projects are completed in approximately 5 weeks. Preliminary results are sent at 2–3 weeks.

TABLE: AKT SUBSTRATE MOTIF PHOSPHOSCAN® FINAL RESULTS, SILAC
Antibody: Phospho-Akt Substrate (RXRXXX/T) (11087) Rabbit mAb #9614
Study Design: Human squamous cell carcinoma (H1703) cell line; Trypsin Digest
Treatments: Untreated (heavy), Wortmannin (Light)

Legend: * - phosphorylation; # - oxidized methionine; § - published site

Rank	Protein Name	Description	Phosphorylation Site	Peptide
1	Akt1	aktlike nucleotidyl transferase 1	S210	YVRLPS*GGKARFPL
2	Akt1	aktlike nucleotidyl transferase 1	S212	TVSLPSS*GKARFPL
3	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
4	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
5	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
6	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
7	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
8	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
9	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
10	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
11	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
12	Akt1	aktlike nucleotidyl transferase 1	S1782	YVSLPSS*GKARFPL
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