

PhosphoScan®, PTMScan® technology for phosphorylation proteomics, uses proprietary phospho-motif and PTM antibodies to enrich tyrosine, serine, or threonine phosphorylated peptides from enzyme digested samples prior to LC-MS/MS analysis. Antibody-based methods provide a more focused approach than other methods, like IMAC, for phosphopeptide enrichment. (IMAC service is also offered by CST.)

PhosphoScan® technology can be used for:

- Novel kinase substrate identification
- Pathway profiling
- Biomarker discovery
- Target validation

Features and Benefits

- PTMScan methods enable focused discovery of even low abundance S/T/Y phosphosites of interest.
- PTMScan technology can be applied to many biological systems and species to encompass diverse research experiments.
- Experienced CST scientists provide technical support throughout the PTMScan workflow to facilitate research progress.

Products and Services

KinomeView® Profiling Kit #9812

PTMScan® Phospho-Akt Substrate Motif mAb 1 (RXXS*/T*) Kit #5561

PTMScan® Phospho-Akt Substrate Motif mAb 2 (RXRXXS*/T*) Kit #5563

PTMScan® Phospho-AMPK Substrate Motif (LXRXS*/T*) Kit #5564

PTMScan® Phospho-ATM/ATR Substrate Motif (pSQ) Kit #12267

PTMScan® Phospho-CK2 Substrate Motif (S*/T*/DXE) Kit #12170

PTMScan® Phospho-MAPK Substrate Motif [PXpTP] Kit #14990

PTMScan® Phospho-MAPK/CDK Substrate Motif (PXS*P and S*PXK/R) Kit #4652

PTMScan® Phospho-PKA Substrate Motif (RRXS*/T*) Kit #5565

PTMScan® Phospho-Ser-Pro-Pro Motif [pSPP] Kit #14989

PTMScan® Phospho-ST*P Motif (ST*P) XP® Rabbit mAb Kit #5566

PTMScan® Phospho-T*PP Motif (T*PP) XP® Rabbit mAb Kit #5567

PTMScan® Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit #8803 (10 assays)

PTMScan® Pilot Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit #14478 (3 assays)

KinomeView® Profiling Service

Serine/Threonine PhosphoScan® Proteomics Services

Tyrosine PhosphoScan® Proteomics Services

PTMScan® Direct Services

IMAC Enrichment Service

For additional information on PTMScan phosphorylation proteomics visit: www.cellsignal.com/phosphoscan

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Complementary Phosphopeptide Enrichment Strategies

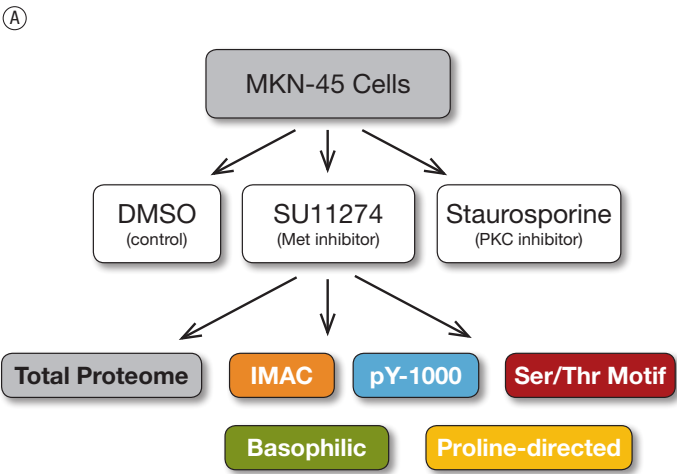
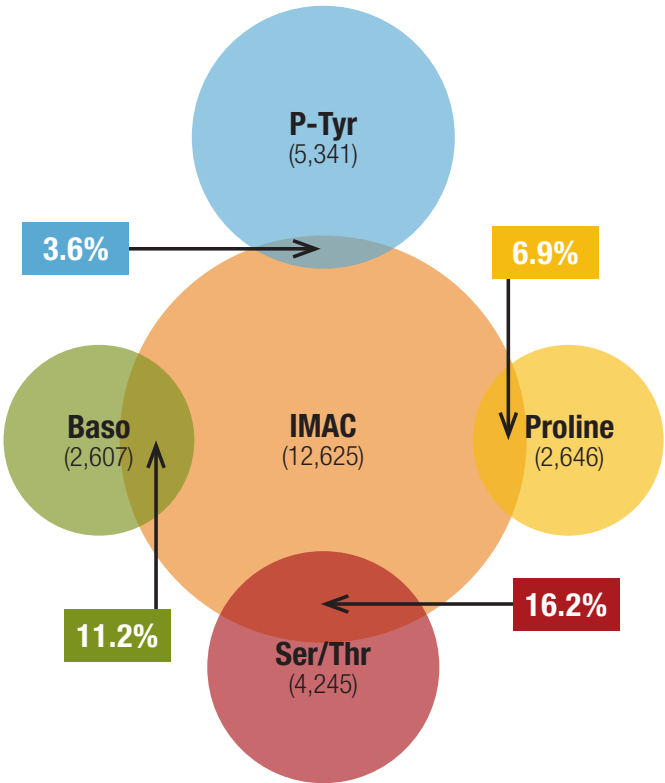
An alternative to antibody-based enrichment of phosphopeptides before LC-MS/MS is immobilized metal affinity chromatography (IMAC), which captures negatively charged phosphopeptides using positively charged metal ions.

PTM profiling of drug response in human gastric carcinoma cells (MKN-45) using antibody and metal affinity-based phosphopeptide enrichment.

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

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 of the overlap between motif antibody and IMAC enrichment of phosphopeptides from MKN-45 cells. Comparisons were made on unique proteins/sites. Antibody mixes were prepared as outlined in the table (B) below.



(B)

Antibody	Motif	pY	Baso	Pro	Ser/Thr
Phosphotyrosine	Y	●			
Akt Substrate	RXX(S/T)		●		●
Akt Substrate	RXRX(S/T)		●		●
AMPK/PKD Substrate	LXRX(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)				●

MKN-45 cells were treated and profiled as outlined (A).
Combinations of motif antibodies were used to enrich phosphopeptides as indicated (B).