Video Article

PTMScan - Proteomics of Post-translational Modifications - ADVERTISEMENT

Jeffrey C. Silva[1], Charles L. Farnsworth[1], Xiaoying Jia[1], Matthew P. Stokes[1], Albrecht Moritz[1], Ailan Guo[1], Roberto D. Polakiewicz[1], Michael J. Comb[1]

[1]Site Discovery Group and PhosphoScan Services Group, Cell Signaling Technology, Inc.

URL: http://www.jove.com/video/2849
DOI: doi:10.3791/2849

Keywords: phosphorylation, proteomics, acetylation, ubiquitinylation, mass spectrometry, motif antibody, immunoprecipitation, phospho-tyrosine, cancer, profiling, PTM, post-translational modification

Date Published: 8/15/2011

Citation: Silva, J.C., Farnsworth, C.L., Jia, X., Stokes, M.P., Moritz, A., Guo, A., Polakiewicz, R.D., Comb, M.J. PTMScan - Proteomics of Post-translational Modifications - ADVERTISEMENT. *J. Vis. Exp.* (), e2849, doi:10.3791/2849 (2011).

Abstract

PTMScan® Technology from Cell Signaling Technology (CST) combines proprietary methodologies for antibody-based peptide enrichment with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantitatively profile post-translational modifications (PTMs) such as phosphorylation (PhosphoScan®), ubiquitination (UbiScan®) and acetylation (AcetylScan®).

PTMScan® Technology employs CST™ Motif Antibodies to separate predefined groups of post-translationally modified peptides from unmodified peptides in digested cell extracts. This immunoaffinity purification technique is combined with LC-MS/MS to identify and quantify changes in modified peptide abundance. Motif antibodies are available to specifically bind phospho (Tyr, Ser, Thr)-peptides, acetylated peptides, or ubiquitin-tagged peptides, enabling the quantitative profiling of the phosphorylation, acetylation, or ubiquitination status of the cell.

MAPK, mTOR and the PI3K/Akt pathways are key signaling pathways activated downstream of oncogenic receptor tyrosine kinases (RTKs). All of these pathways activate AGC kinase family members, including Akt, RSK, and p70 S6 kinases, whose protein substrates are phosphorylated at the RxRxxS/T motif.

In a recent phosphoproteomic study authored by scientists at CST (Sci. Signal. (2010) 24;3(136):ra64), over 300 putative substrates for these AGC family kinases were identified in three cell lines, each driven by either EGFR, c-Met, or PDGFR. The experimental approach employed PhosphoScan® Technology using the RxRxxS/T Motif Antibody as an affinity reagent to selectively immunoprecipitate phosphorylated substrates of Akt, RSK, and p70 S6 kinases. Use of targeted cancer drugs with inhibitors specific for PI3K, mTOR, and MEK allowed for mapping of the signaling network downstream of these RTKs. Substrates included proteins involved in many cellular functions, including scaffolding, protein stability, metabolism, trafficking, and motility.

Video Link

The video component of this article can be found at http://www.jove.com/video/2849/

