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PTMScan direct: Quantitative profiling of critical signaling pathways using immunoaffinity purification and LC-MS/MS

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Abstract

Core signaling pathways regulate diverse cellular processes as well as disease states such as oncogenesis or neurodegeneration. Proteomic methods designed to interrogate these pathways are therefore desirable to better understand cellular signaling and disease biology. PTMScan Direct is an antibody-based method combining immunoaffinity enrichment with LC-MS analytics for the identification and quantification of post-translationally modified peptides from critical signaling pathways. The PTMScan Direct method is compatible with both label-free quantitation as well as labeling methods such as SILAC. A total of six PTMScan Direct reagents have been developed that target diverse biological areas including: Ser/Thr Kinase (1) and Tyr Kinase activity (2), the PI3K-Akt pathway (3), DNA Damage and Cell Cycle Regulation (4), Apoptosis and Autophagy Pathways (5), as well as a Multipath way Reagent that targets core signaling proteins from a number of critical regulatory pathways (6). These reagents have been validated for use in human and mouse cell lines, tissues, and xenografts. Each reagent targets hundreds of unique post-translationally modified peptides in a single LC-MS/MS run. The technology has been used to profile cellular responses to a variety of stimuli including drug treatments such as Imatinib or Gefitinib, DNA damaging agents such as UV light, and growth factors such as EGF. In addition, the approach has also been used to assess changes in post-translationally modified peptide abundanceamongvarious mouse tissues such as liver, brain, and embryo to reveal unique signatures of tissue specific signaling. PTMScan Direct is broadly applicable to any experimental system in which quantitative profiling of specific critical signaling molecules is desirable.