Multiplexed cellular signaling pathway profiling through targeted peptide immunoenrichment and quantitative analysis by LCMS

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INTRODUCTION

It is clear that robust methods are needed to monitor the activity of cellular signaling pathways in all diseases and in all steps of drug discovery and development. Current methods such as IMAC are often utilized for broad phosphorylation enrichment, but sample complexity results in stochastic peptide identification. Bead-based arrays and ELISA assays are available only to a select few commonly studied targets, and with limited multiplexing.

METHODS

We have developed the PTMScan® Multi-Pathway Enrichment Kit to bridge this gap between highly targeted assays and broad, complex samples, providing pathway-based peptide enrichment upstream of LCMS analysis. This reagent contains an array of site-specific antibodies representing key signaling nodes from multiple pathways, including cell cycle and checkpoint control, AKT/PI3K signaling, T-cell and B-cell receptor signaling, MAPK and JNK cascades, and many others. To profile pathway activity, proteins are proteolytically digested, and enrichment is performed at the peptide level. Enriched peptides and phosphopeptides are analyzed through data-dependent LC-MS/MS for total pathway profiling or through targeted MS analysis in multiplexed assay format.

RESULTS

We have used the PTMScan® Multi-Pathway Enrichment Kit to quantify peptides from mouse tissues and multiple human cell lines. Using data-dependent LC-MS/MS, thousands of peptides are identified representing hundreds of sites of phosphorylation, enabling measurement of activity of dozens of signaling pathways. We compare use of this reagent with IMAC phosphopeptide enrichment to assess breadth and depth of coverage of key signaling nodes. This enrichment reagent has also been utilized for peptide purification upstream of targeted LCMS analysis, quantifying dozens of peptides with atomic sensitivity.

CONCLUSIONS

• PTMScan® Multi-Pathway Enrichment Kit enables identification of thousands of target peptides in defined signaling pathways
• Target-specific enrichment significantly reduces required instrument time
• Pathway-based enrichment enables hypothesis-driven, targeted studies with data acquisition and quantitative flexibility

REFERENCES


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