Development of control peptides for immunoaffinity-based enrichment of posttranslationally modified peptides

INTRODUCTION

Posttranslational modifications (PTMs) are important elements of many biological processes. A key aspect to experiments that aim to identify and quantify PTM sites is the use of appropriate techniques such as immunoaffinity purification (IAP) or immobilized metal affinity chromatography (IMAC) to enrich for PTMs prior to liquid chromatography-mass spectrometry (LCMS) analysis. To facilitate IAP and IMAC workflows, we developed a series of synthetic peptides that contain a specific PTM and a stable heavy isotope. The goal of such synthetic peptides is to be added into samples during PTM enrichment and assayed as an internal control for workflow performance and reproducibility.

METHODS

We based the synthetic control peptide sequences on naturally occurring PTM sites consistently identified in our department’s internal research using PTMScan® IAP and IMAC workflows.

Peptides were synthesized using FMOC protected amino acids (Glyco Protein Technologies) and Carboxyl Resins (EMD) on a CEM Multipep 2 peptide synthesizer. Isotopically labeled FMOC protected amino acids (13C, 15N-Lysine or 13C, 15N, Arginine) were sourced from Cambrex Isotope Labs. pHBTU (Chem-Impex) and 4-Methylmorpholine (EMD) were used for activation of the amino acids. After synthesis, the peptides were cleaved with 87.5% trifluoroacetic acid (TFA), 5% Dimethyl sulfoxide, 2.5% H2O, 2.5% Triisopropylsilane, 2.5% Ethanedithiol. Cleaved peptides were precipitated twice with Diethyl ether. Crude peptides were purified using Reverse Phase C18 HPLC and an accurate peptide concentration was determined by amino acid analysis. Peptide purity and sequence were confirmed by LCMS. The final peptide stocks were reconstituted in 25% Acetonitrile (MeCN), 74.9% H2O, and 0.1% TFA.

To assess the optimal concentration range for use, serial dilution of Control Peptides at 10 μL volume was added to sample peptides (e.g. mouse liver triplicates) at a final volume of 1.5-1.3 mL prior to PTM enrichment. Washes, elution, and desalting were performed as specified in the respective PTMScan® workflows.

CONCLUSIONS

We identified a set of synthetic Control Peptides that can be quantitatively enriched by the respective PTMScan® workflows without negative impact on the analysis of endogenous modified peptides. Such peptides can be used to assess the reproducibility of PTM enrichment steps independent of upstream sample peptide preparation steps.

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Barry M. Zee, Hayley J. Peckham, Charles L. Farnsworth, Alissa J. Nelson, Kathryn E. Abell, Vicky Yang, Jeffrey Knott, Matthew P. Stokes

Cell Signaling Technology, Inc., Danvers MA 01923

Control Peptide & PTMScan® Workflow

Control Peptide Recovery from biological samples using PTMScan® reagents

Control Peptide Quantification Range

Control Peptide Reproducibility

Table 1: List of all Control Peptides developed and demonstrated compatibility with existing respective PTM enrichment workflows, including both agarose and magnetic bead-based formats (i.e. PTMScan® HS and IMAC). Each set of Control Peptides for a particular PTM is comprised of at least 3 unique peptide components.