

Post-Translational Modification Analysis

By Liquid Chromatography—Tandem Mass Spectrometry

Introduction

Proteins are subjected to a wide variety of covalent modifications after translation. Many of these post-translational modifications (PTMs) are critical to the protein's function. The common PTMs includes Phosphorylation, Ubiquitination, Methylation, Acetylation and Glycosylation. Our accumulated experiences enabled us to develop reliable workflows using available tools and technologies for the identification of PTMs.

Poochon's protein PTM analysis services are designed for identification of PTMs, including S/T/Y phosphorylation, R/K methylation, K/H acetylation, K ubiquitination, etc., from crude, partially purified, or purified protein samples by using a combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS).

Specifications

- **Method** – Protease digestion and LC-MS/MS
- **Key Instruments** – Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer, Thermo Scientific™ UltiMate™ 3000 RSLCnano System
- **Detection Limit** – Target protein: $\geq 0.1 \mu\text{g}$ for PTM analysis; $\geq 1 \text{ ng}$ for protein identification
- **Acceptable Samples** – Purified protein in solution or dried ($\geq 0.1 \mu\text{g}/\text{sample}$), partially purified samples ($\geq 1 \mu\text{g}/\text{sample}$), tissue/cell lysate ($\geq 100 \mu\text{g}/\text{sample}$), cell pellet (> 1 million cells/sample), solid tissue ($\geq 50 \text{ mg}/\text{sample}$)
- **Turnaround Time** – Typically, reports are available within 5 business days of sample receipt

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Schematic of Procedure Workflow

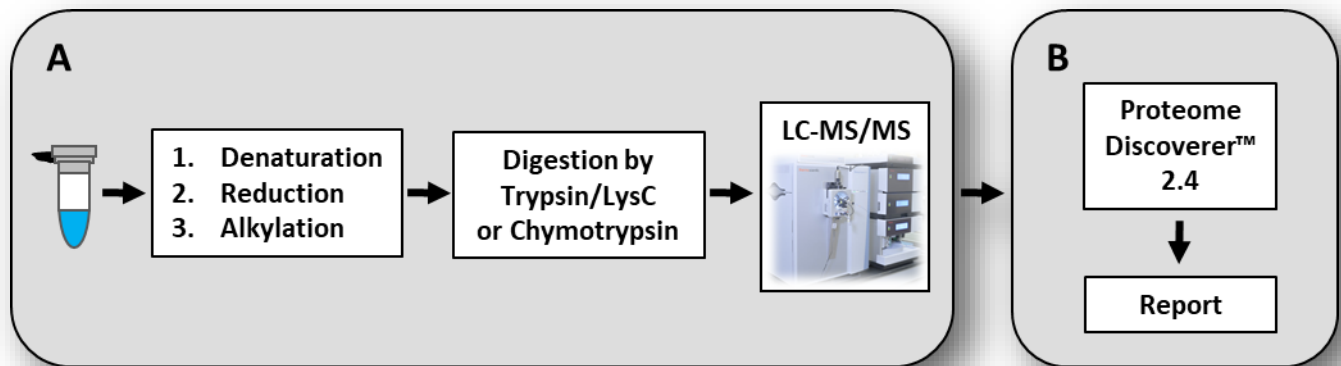


Figure 1:

A) Workflow for PTM and Protein ID analysis. *Note: LC-MS/MS = liquid chromatography and tandem mass spectrometry*

B) Bioinformatic analysis approach used for protein peptide and PTM identification of datasets.

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Example One

Identification of conversion of the proline (P) residue at amino acid 45 to glutamic acid (E) in human protein Peroxiredoxin-6

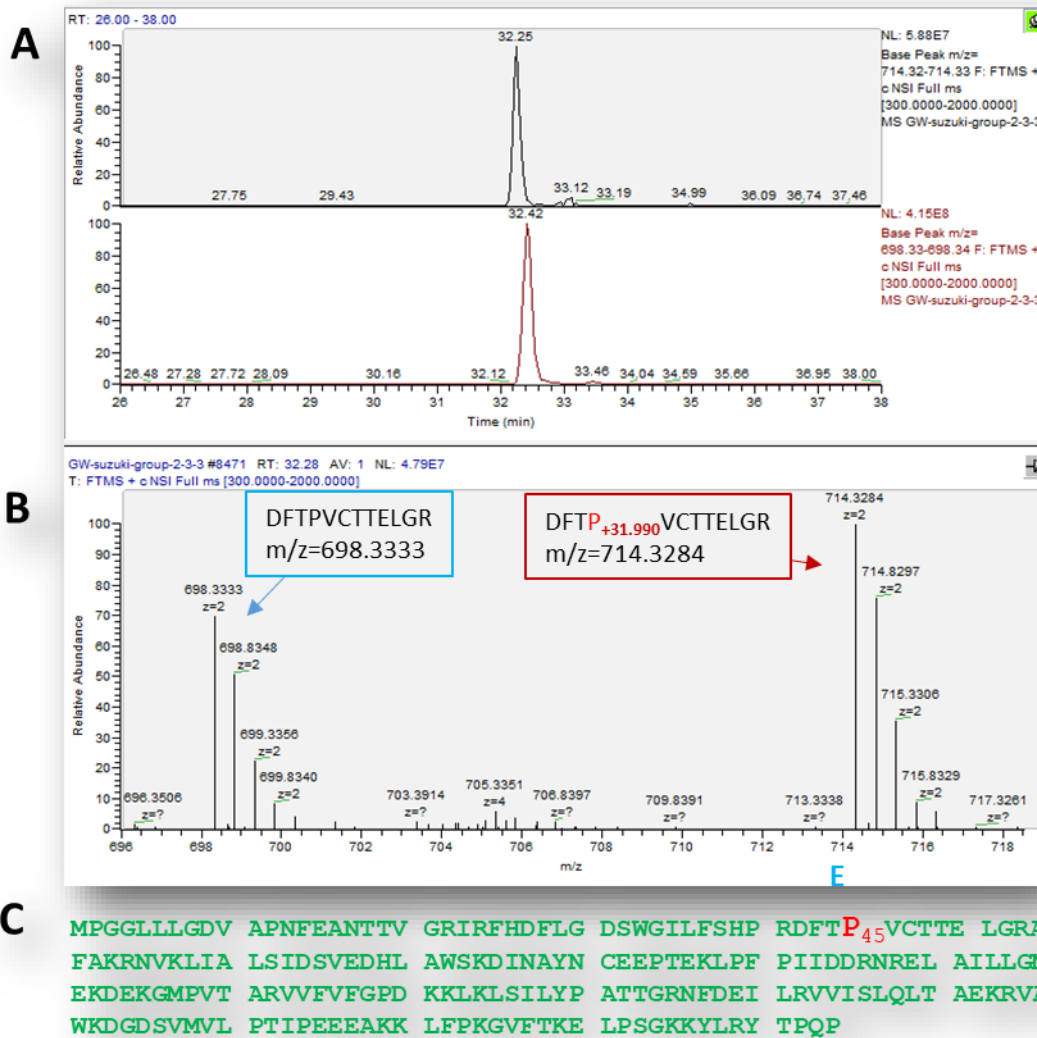


Figure 1: Nano LC-MS/MS verification of conversion of Pro 45 to Glu at PRDX6 (DFTP^{+31.990}VCTTELGR). **A)** Extracted ion chromatograms of PRDX6 peptide (DFTP^{+31.990}VCTTELGR, +2 charge, m/z=714.33) (top), and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) (bottom). Both peptides were eluted at the same retention time and are from affinity-enriched cultured human cell extract using anti-PRDX6 antibody. **B)** High resolution MS spectra of co-elution of peptides (DFTP^{+31.990}VCTTELGR, +2 charge, m/z=714.33) (right), and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) (left). **C)** Protein sequence

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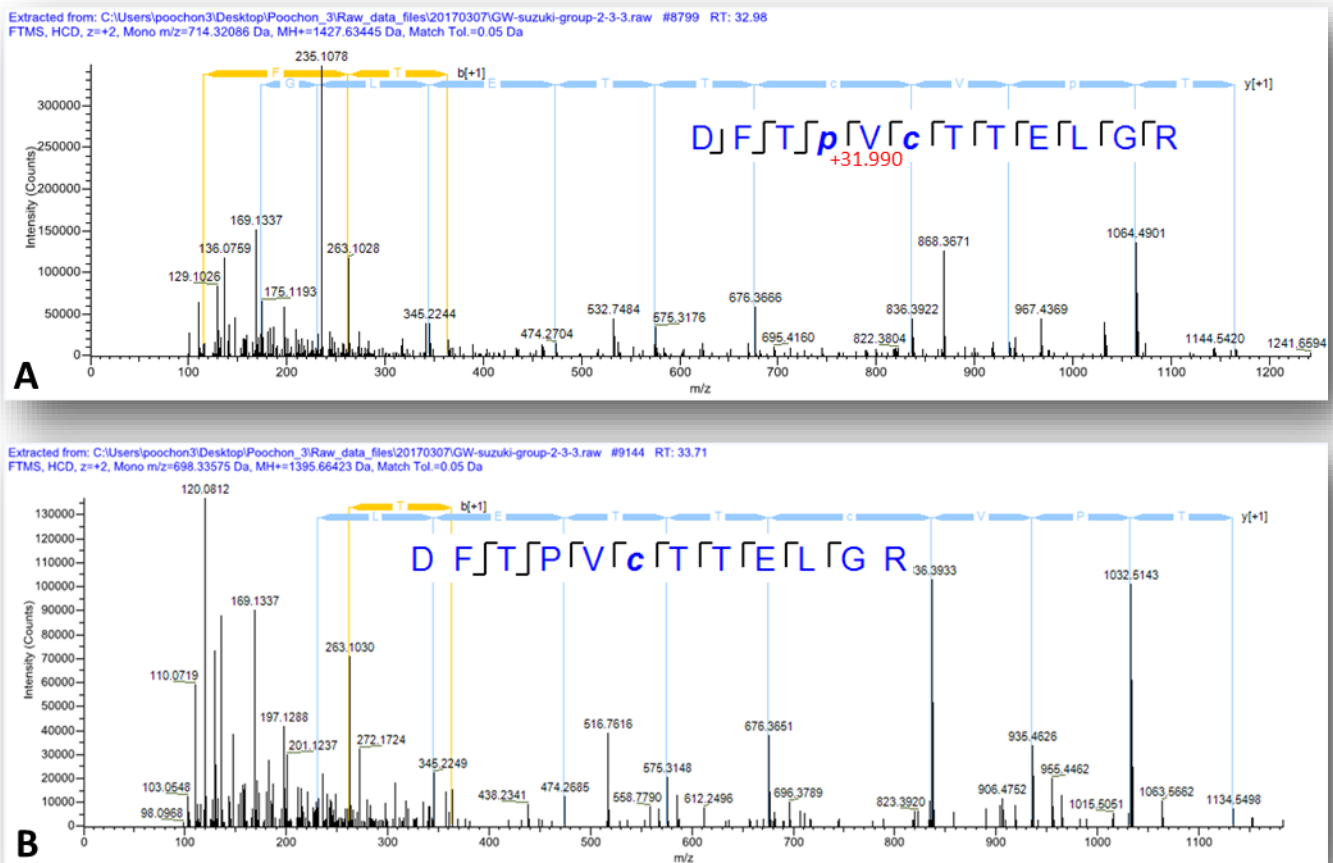


Figure 2: Nano LC-MS/MS verification of conversion of proline 45 to glutamic acid at PRDX6 (DFTP^{+31.990}VCTTELGR). **A)** High resolution MS/MS spectra of PRDX6 Glu conversion peptide (DFTP^{+31.990}VCTTELGR). **B)** High resolution MS/MS spectra of PRDX6 Pro 45 peptide (DFTP^{+31.990}VCTTELGR).

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Example Two

PTM Analysis of Factor VIII

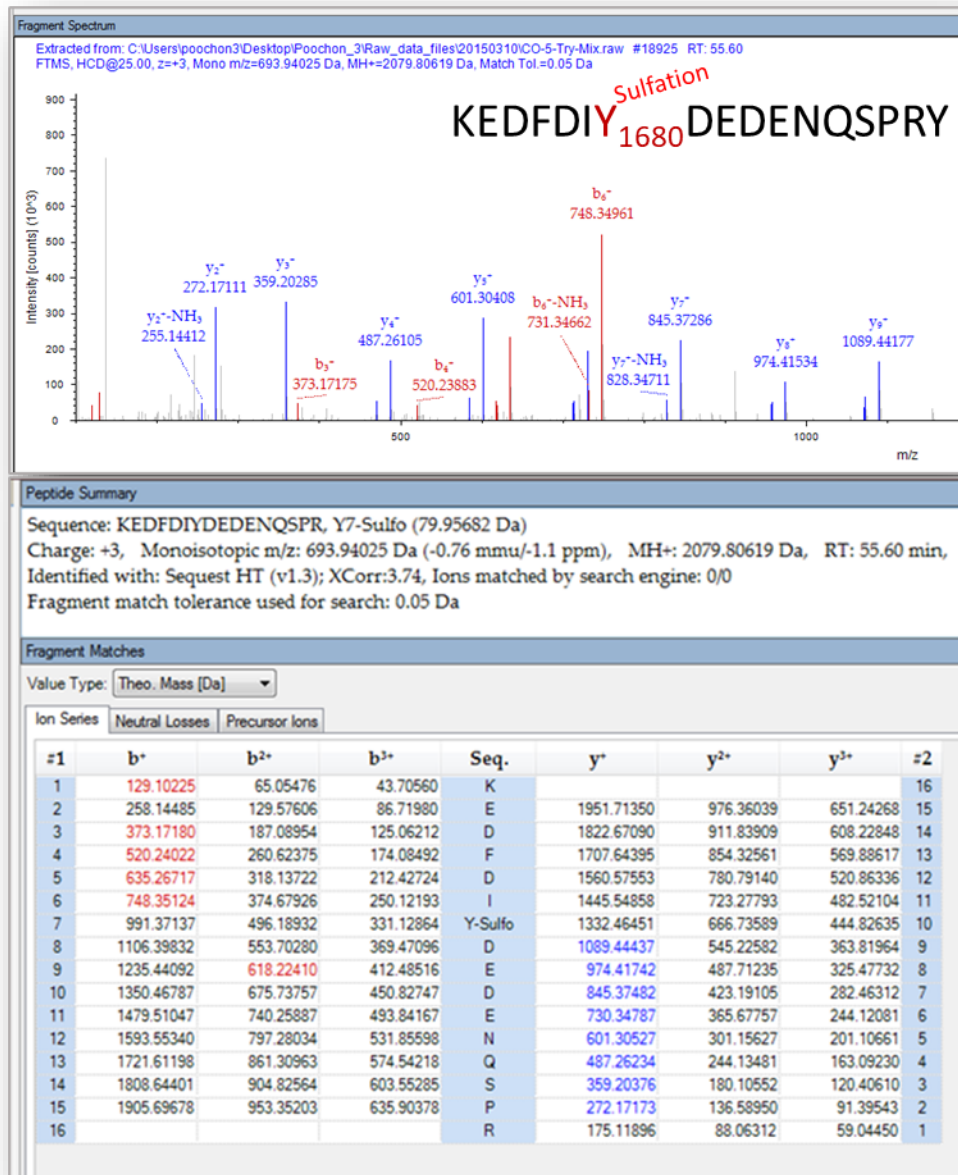


Figure 3: Analysis of tyrosine sulfation of Factor VIII by LC-MS/MS. High resolution MS/MS spectra of one peptide (KEDFDIY₁₆₈₀DEDENQSPRY) with sulfation on Y1680.

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Example Two

PTM Analysis of Factor VIII

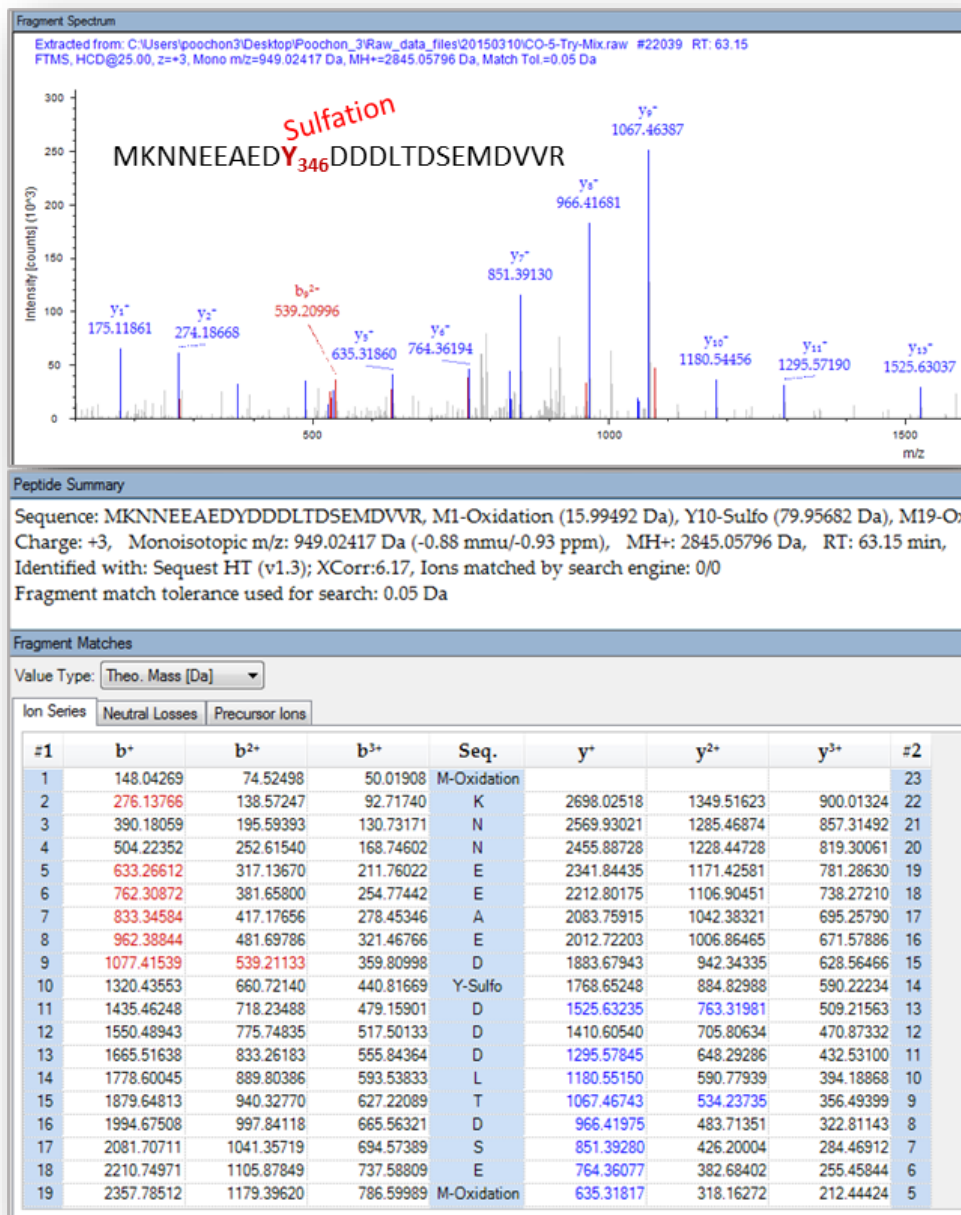


Figure 4: Analysis of tyrosine sulfation of Factor VIII by LC-MS/MS. High resolution MS/MS spectra of one peptide (MKNNEEAEDY₃₄₆DDDLTDSEMDVVR) with sulfation on Y346.

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Example Three

S1943 Phosphorylation Analysis of MYH9

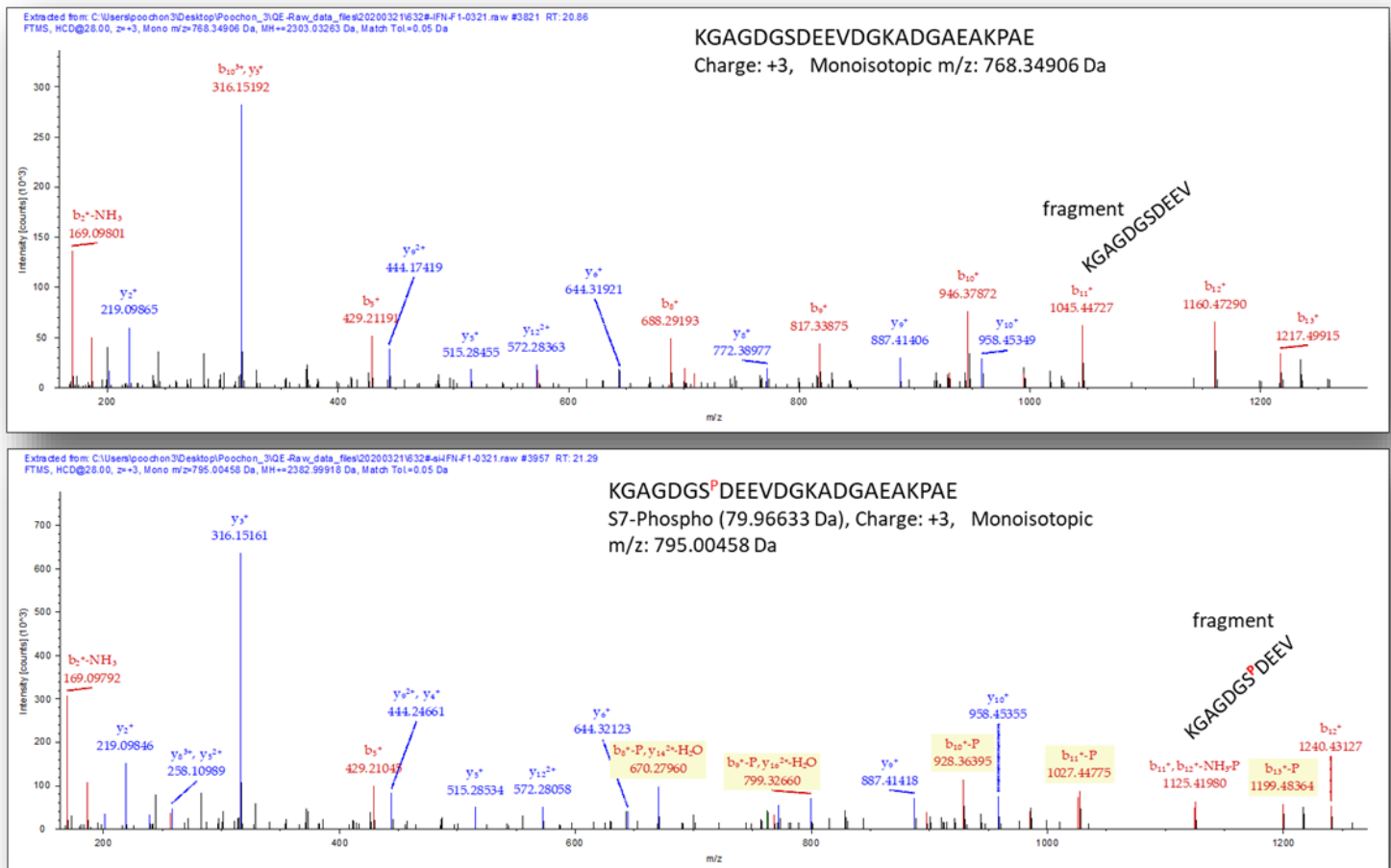


Figure 5: Nano LC-MS/MS verification of MYH9 S1943 phosphorylation. **A)** High resolution MS/MS spectra of MYH9 peptide (KGAGDGSDEEVDGKADGAEAKPAE). **B)** High resolution MS/MS spectra of MYH9 peptide (KGAGDGS^PDEEVDGKADGAEAKPAE, S7-Phospho (79.96633 Da).