

# **Absolute Quantitation of Glycosylation Site Occupancy Using Isotopically Labeled Standards and LC-MS**

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## **Supplementary Material**

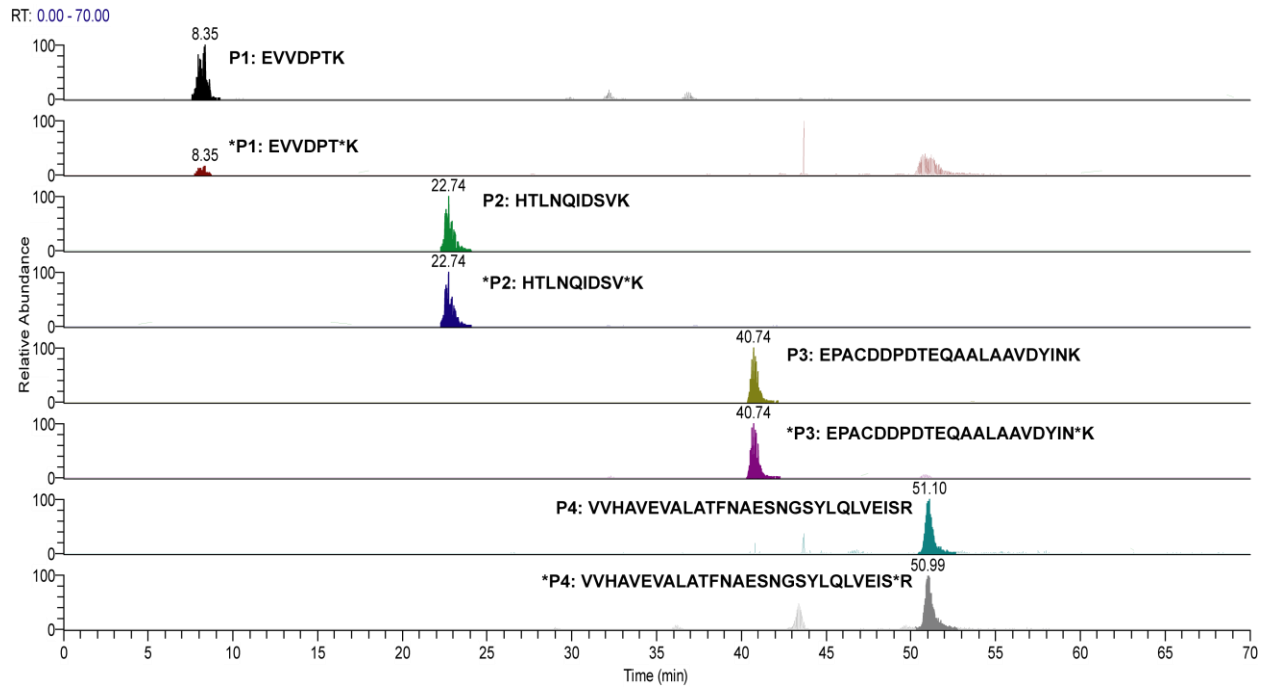
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**Supplementary Table 1.** Peptide sequences contained in fetuin and their heavy isotope-labeled peptide standard counterparts. Each standard has <sup>13</sup>C and <sup>15</sup>N labeled on the terminal lysine or arginine residue. The potential glycosylation site on P4 is labeled in blue.

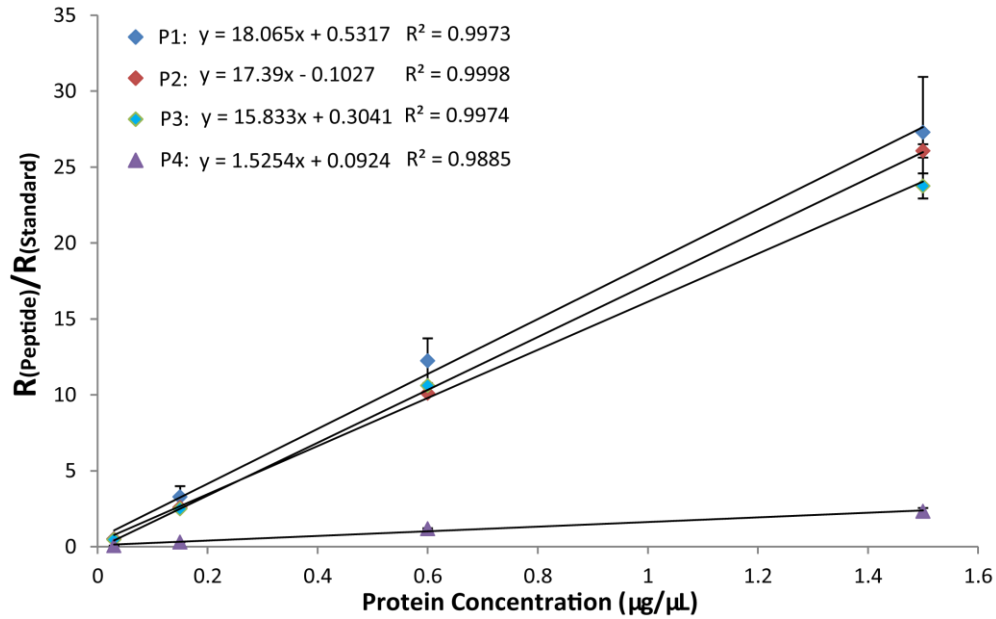
Peptide	Sequence	Monoisotopic Mass	Charge State	Precursor <i>m/z</i>
P1	EVVDPTK	786.4123	1+	787.4196
*P1	EVVDPT*K	794.4265	1+	795.4338
P2	HTLNQIDSVK	1153.6091	2+	577.8118
*P2	HTLNQIDSV*K	1161.6233	2+	581.8189
P3	EPACDDPDTEQAALAAVDYINK	2405.0693	3+	802.6970
*P3	EPACDDPDTEQAALAAVDYIN*K	2413.0835	3+	805.3684
P4	VVHAVEVALATFNAESNGSYLQLVEISR	3015.5665	3+	1006.1961
*P4	VVHAVEVALATFNAESNGSYLQLVEIS*R	3025.5748	3+	1009.5322

**Supplementary Table 2.** The percent glycosylation site occupancy (%) determined by comparing the quantitation results of different normal peptides (P1-3) against the non-glycosylated peptide (P4). Fetuin samples of varied concentrations (0.03-1.5  $\mu\text{g}/\mu\text{L}$ ) were analyzed.

Concentration ( $\mu\text{g}/\mu\text{L}$ )	C1 = 0.03	C2 = 0.15	C3 = 0.6	C4 = 1.5
P1 & P4	86.2 $\pm$ 2.8	90.8 $\pm$ 2.5	90.3 $\pm$ 0.7	91.4 $\pm$ 1.0
P2 & P4	86.2 $\pm$ 0.8	88.8 $\pm$ 0.3	88.4 $\pm$ 0.7	91.1 $\pm$ 0.9
P3 & P4	86.4 $\pm$ 2.4	88.4 $\pm$ 0.4	89.0 $\pm$ 0.4	90.2 $\pm$ 0.9



**Supplementary Figure 1.** Representative extracted ion chromatograms of the tryptic peptides generated from fetuin and their spiked heavy isotope-labeled standards. The peak area of the monoisotopic peak ( $m/z$  values listed in Supplementary Table 1) of each peptide is used in the quantitative analysis.



**Supplementary Figure 2.** The ratio of each fetuin peptide's (P1-4) signal response (peak area) over the signal response of the corresponding heavy isotope labeled peptide standard (\*P1-4), was plotted against the fetuin concentration (0.03-1.5  $\mu\text{g}/\mu\text{L}$ ), to construct the four calibration curves shown in this figure. Each sample was spiked with the same amount (50 pmol) of the four heavy isotope labeled peptide standards (\*P1-4). Each dilution was analyzed in triplicate.