Store at -20°C

PTMScan® Control Peptides Phospho-Enrichment IMAC

#59524

1 vial

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www.cellsignal.com/support

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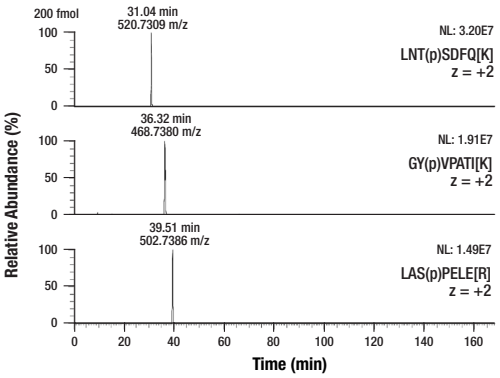
For Research Use Only. Not For Use In Diagnostic Procedures.

Number	Peptide	Precursor mass ($M+H^+$)	Recommended m/z to monitor
1	LNT(p)SDFQ[K]	1040.45397 m/z	520.73062 m/z (z = +2)
2	GY(p)VPATI[K]	936.468161 m/z	468.73772 m/z (z = +2)
3	LAS(p)PELE[R]	1004.46877 m/z	502.73802 m/z (z = +2)

Peptides included in the PTMScan® Control Peptides Phospho-Enrichment IMAC mix. All peptides are stable-isotope labeled, designated by bracketed R or K, and contain a phosphate group designated by parentheses.

Description: The PTMScan® Control Peptides Phospho-Enrichment IMAC enable quality control of enrichment performance using PTMScan® workflows. These synthetic peptides contain a specific post-translational modification (PTM) that can be enriched by the associated PTMScan® beads, as well as a stable heavy isotope that can be distinguished from endogenous peptides by the mass spectrometer.

Background: Immobilized metal affinity chromatography, or IMAC, has been widely used to enrich proteins and peptides from biological samples by binding to clusters of negative charge. Divalent transition metal ions Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} are often used to purify proteins rich in poly-Histidine or Cysteine as well as proteins with metal affinity. Trivalent metal ions, Fe^{3+}, Ga^{3+}, Al^{3+}, as well as Ti^{4+} and Zr^{4+} are commonly used for phosphopeptide enrichment for proteomic studies (1). Iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) are used for chelating the metal ions to agarose-coated beads. In comparison studies, NTA has been shown to perform better than IDA at selectively capturing and identifying more phosphopeptides. Ga^{3+} and Fe^{3+} are comparable with respect to the number of phosphopeptides identified (2). Compared to metal oxide affinity chromatography (MOAC) using TiO_2, Fe^{3+} IMAC performed marginally better with TiO_2 having a preference for acidic phosphopeptides (pI > 4) relative to Fe^{3+} which prefered less acidic peptides (pI < 4) (3). The PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads #20432 offer an efficient tool for phosphopeptide enrichment with little or no bias for phospho-residue context. They can be employed independently or in conjunction with immunoaffinity based enrichment to complement any PTMScan® LC-MS/MS proteomic study.



Extracted ion chromatograms of PTMScan® Control Peptides Phospho-Enrichment IMAC added at supplied concentration (1X at 200 fmol) to secondary digest mouse liver peptides prior to enrichment using PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads #20432. Desalted peptides were analyzed on Q Exactive™ mass spectrometer and resolved using a 150 min reversed phase gradient from 7.5% to 32% acetonitrile on a C18 column. The peak corresponding to the specific Control Peptide is marked with retention time and observed precursor mass, with peak height reported as the normalized level (NL) for each row per panel.

Storage: This product is stable for 24 months when stored at -20°C. *Aliquot to avoid multiple freeze/thaw cycles.*

Please visit www.cellsignal.com for a complete listing of recommended complementary products.

Directions for Use:

Use with Cell Signaling Technology's PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads #20432 kit protocol from the incubation step. Because the optimal amount of PTMScan® Control Peptides Phospho-Enrichment IMAC for each user's experiments will depend on unique factors, such as mass spectrometer sensitivity, users may dilute these control peptides as needed in the appropriate solvent.

1. Aliquot PTMScan® Control Peptides Phospho-Enrichment IMAC for storage as single-use units at -20°C or proceed to immediate usage.
2. Perform secondary trypsin digest of sample peptides in the appropriate buffer and volume for two hours at 37°C, e.g., 50 µL of buffer containing 5% acetonitrile, 50 mM ammonium bicarbonate, and 100 ng/µL trypsin.
3. Add 0.95 mL Loading Buffer (0.1% trifluoroacetic acid, 85% acetonitrile) to the digested solution and vortex.
4. Clear sample peptides by centrifugation.
5. Transfer clarified sample peptides to tubes containing washed IMAC magnetic beads.
6. Add 10 µL of PTMScan® Control Peptides Phospho-Enrichment IMAC to tubes containing beads and sample peptides and mix well.
7. Continue with IMAC workflow at the 30 minute incubation step.
8. Detect PTMScan® Control Peptides Phospho-Enrichment IMAC in the LCMS data file.

Background References:

(1) Block, H. et al. (2009) *Methods Enzymol* 463, 439-73.

(2) Ficarro, S.B. et al. (2009) *Anal Chem* 81, 4566-75.

(3) Yue, X. et al. (2015) *Anal Chem* 87, 8837-44.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.