Store at -20°C

PTMScan® Control Peptides Pan-Methyl Lysine

#35263

1 vial

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

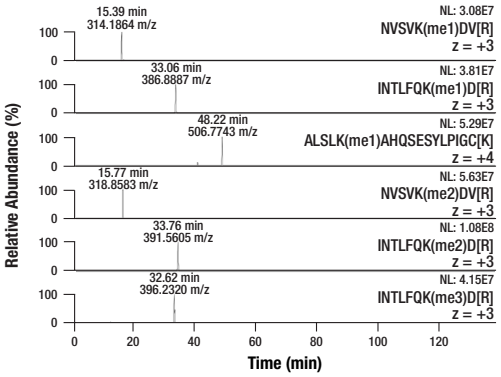
Number	Peptide	Precursor mass (M+H⁺)	Recommended m/z to monitor
1	NVSVK(me1)DV[R]	940.54498 m/z	314.18651 m/z (z = +3)
2	INTLFQK(me1)D[R]	1158.65050 m/z	386.88835 m/z (z = +3)
3	ALSLK(me1)AHQSESYLPIGC[K]	2024.07247 m/z	506.77358 m/z (z = +4)
4	NVSVK(me2)DV[R]	954.56063 m/z	318.85839 m/z (z = +3)
5	INTLFQK(me2)D[R]	1172.66615 m/z	391.56024 m/z (z = +3)
6	INTLFQK(me3)D[R]	1186.68180 m/z	396.23212 m/z (z = +3)

Peptides included in the PTMScan® Control Peptides Pan-Methyl Lysine mix. All peptides are stable-isotope labeled, designated by bracketed R or K, and contain a mono-methyl, di-methyl, or tri-methyl group designated by parentheses. Cysteine has been modified with a carbamidomethyl group.

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

Background: Methylation of lysine residues is a common regulatory post-translational modification (PTM) that results in the mono-, di-, or tri-methylation of lysine at ε-amine groups by protein lysine methyltransferases (PKMTs). Two PKMT groups are recognized based on structure and catalytic mechanism: class I methyltransferases or seven β strand enzymes, and SET domain-containing class V methyltransferases. Both use the methyl donor S-adenosyl-L-methionine to methylate histone and non-histone proteins. Class I methyltransferases methylate amino acids, DNA, and RNA (1,2). Six methyl-lysine-interacting protein families are distinguished based on binding domains: MBT, PHD finger, Tudor, PWWP, WD40 repeat, and chromodomains. Many of these display differential binding preferences based on lysine methylation state (3). KDM1 subfamily lysine demethylases catalyze demethylation of mono- and di-methyl lysines, while 2-oxoglutarate-dependent JmjC (KDM2-7) subfamily enzymes also modify tri-methyl lysine residues (4).

Most PKMT substrates are histone proteins and transcription factors, emphasizing the importance of lysine methylation in regulating chromatin structure and gene expression. Lys9 of histone H3 is mono- or di-methylated by G9A/GLP and tri-methylated by SETDB1 to activate transcription. JHDM3A-mediated demethylation of the same residue creates mono-methyl Lys9 and inhibits gene transcription (5). Tumor suppressor p53 is regulated by methylation of at least four sites. p53-mediated transcription is repressed following mono-methylation of p53 at Lys370 by SMYD2; di-methylation at the same residue further inhibits p53 by preventing association with 53BP1. Concomitant di-methylation at Lys382 inhibits p53 ubiquitination following DNA damage. Mono-methylation at Lys382 by SET8 suppresses p53 transcriptional activity, while SET7/9 mono-methylation at Lys372 inhibits SMYD2 methylation at Lys370 and stabilizes the p53 protein. Di-methylation at Lys373 by G9A/GLP inhibits p53-mediated apoptosis and correlates with tri-methylation of histone H3 Lys9 at the p21 promoter (1,6). Overexpression of PKMTs is associated with multiple forms of human cancer, which has generated tremendous interest in targeting protein lysine methyltransferases in drug discovery research.



Extracted ion chromatograms of PTMScan® Control Peptides Pan-Methyl Lysine added at supplied concentration (1X at 200 fmol) to mouse liver peptides prior to immunoaffinity enrichment using PTMScan® Pan-Methyl Lysine Kit #14809. Desalted peptides were analyzed on Q Exactive™ mass spectrometer and resolved using a 120 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 validation data and a complete listing of recommended companion products.

Directions for Use:

Use with Cell Signaling Technology's PTMScan® kit protocol from the Immunoaffinity Purification (IAP) step. Because the optimal amount of PTMScan® Control Peptides Pan-Methyl Lysine for each user's experiments will depend on unique factors, such as mass spectrometer sensitivity, users may dilute these control peptides as needed.

1. Aliquot PTMScan® Control Peptides Pan-Methyl Lysine for storage as single-use units at -20°C or proceed to immediate usage.
2. Resuspend sample peptides in the appropriate buffer and volume, e.g., 1.4 mL of PTMScan® IAP Buffer (1X).
3. Clear sample peptides by centrifugation.
4. Transfer clarified sample peptides to tubes containing IAP beads.
5. Add 10 µL of PTMScan® Control Peptides PanMethyl Lysine to IAP beads and sample peptides and mix well.
6. Continue with PTMScan® or PTMScan® HS workflows at the 2-hour incubation step.
7. Detect PTMScan® Control Peptides Pan-Methyl Lysine in the LCMS data file.

Background References:

(1) Lanouette, S. et al. (2014) *Mol Syst Biol* 10, 724.
(2) Clarke, S.G. (2013) *Trends Biochem Sci* 38, 243-52.
(3) Herold, J.M. et al. (2011) *Curr Chem Genomics* 5, 51-61.
(4) Thinnes, C.C. et al. (2014) *Biochim Biophys Acta* 1839, 1416-32.
(5) Klose, R.J. et al. (2006) *Nature* 442, 312-6.
(6) Yost, J.M. et al. (2011) *Curr Chem Genomics* 5, 72-84.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **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.