Store at -20°C

PTMScan® Control Peptides Asymmetric Di-Methyl Arginine

#51396

1 vial

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

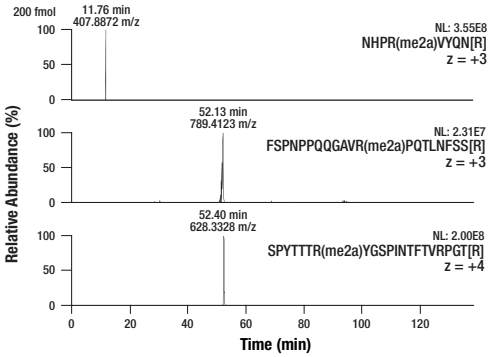
Number	Peptide	Precursor mass (M+H+)	Recommended m/z to monitor
1	NHPR(me2a)VYQN[R]	1221.64748 m/z	407.88735 m/z (z = +3)
2	FSPNPPQQGAVR(me2a)PQTLNFSS[R]	2366.22392 m/z	789.41283 m/z (z = +3)
3	SPYTTTR(me2a)YGSPINTFTVRPGT[R]	2510.30257 m/z	628.33110 m/z (z = +4)

Peptides included in the PTMScan® Control Peptides Asymmetric Di-Methyl Arginine mix. All peptides are stable-isotope labeled, designated by bracketed R, and contain a di-methyl group designated by parentheses.

Description: The PTMScan® Control Peptides Asymmetric Di-Methyl Arginine 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: Arginine methylation is a prevalent PTM found on both nuclear and cytoplasmic proteins. Arginine methylated proteins are involved in many different cellular processes, including transcriptional regulation, signal transduction, RNA metabolism, and DNA damage repair (1-3). Arginine methylation is carried out by the arginine N-methyltransferase (PRMT) family of enzymes that catalyze the transfer of a methyl group from S-adenosylmethionine (AdoMet) to a guanidine nitrogen of arginine (4). There are three different types of arginine methylation: asymmetric dimethylarginine (aDMA, omega-NG,NG-dimethylarginine), where two methyl groups are placed on one of the terminal nitrogen atoms of the guanidine group of arginine; symmetric dimethylarginine (sDMA, omega-NG,N'G-dimethylarginine), where one methyl group is placed on each of the two terminal guanidine nitrogens of arginine; monomethylarginine (MMA, omega-NG-methylarginine), where a single methyl group is placed on one of the terminal nitrogen atoms of arginine. Each of these modifications has potentially different functional consequences. Though all PRMT proteins catalyze the formation of MMA, Type I PRMTs (PRMT1, 3, 4, 6, and 8) add an additional methyl group to produce aDMA, while Type II PRMTs (PRMT5 and 7) produce sDMA. Methylated arginine residues often reside in glycine-arginine rich (GAR) protein domains, such as RGG, RG, and RXR repeats (5). However, PRMT4/CARM1 and PRMT5 methylate arginine residues within proline-glycine-methionine rich (PGM) motifs (6).

In undifferentiated mouse embryonic neural precursors, sDMA histone H4R3 is prevalent, but in later stages of development, both sDMA and aDMA di-methyl H4R3 modifications are detected in post-mitotic neurons and developing oligodendrocytes. This implies that sDMA modifications may be negative epigenetic regulatory events while aDMA modifications may signal epigenetic activation sites (7).



Extracted ion chromatograms of PTMScan® Control Peptides Asymmetric Di-Methyl Arginine added at supplied concentration (1X at 200 fmol) to mouse liver peptides prior to immunoaffinity enrichment using PTMScan® Asymmetric Di-Methyl Arginine Motif [adme-R] Kit #13474. 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 12 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 Asymmetric Di-Methyl Arginine 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 Asymmetric Di-Methyl Arginine 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 Asymmetric Di-Methyl Arginine 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 Asymmetric Di-Methyl Arginine in the LCMS data file.

Background References:

(1) Bedford, M.T. and Richard, S. (2005) *Mol Cell* 18, 263-72.
(2) Pahlich, S. et al. (2006) *Biochim Biophys Acta* 1764, 1890-903.
(3) Bedford, M.T. and Clarke, S.G. (2009) *Mol Cell* 33, 1-13.
(4) McBride, A.E. and Silver, P.A. (2001) *Cell* 106, 5-8.
(5) Gary, J.D. and Clarke, S. (1998) *Prog Nucleic Acid Res Mol Biol* 61, 65-131.
(6) Cheng, D. et al. (2007) *Mol Cell* 25, 71-83.
(7) Chittka, A. (2010) *PLoS One* 5, e13807.

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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.