Store at -20°C

PTMScan® Control Peptides Succinyl-Lysine

#30299

1 vial

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

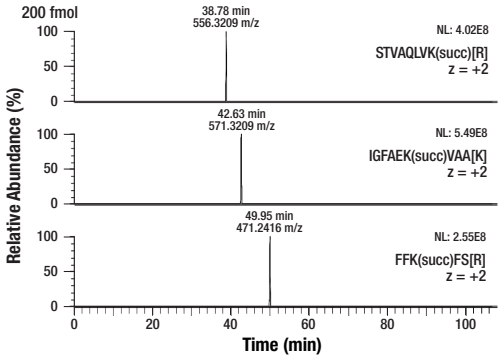
Number	Peptide	Precursor mass ($M+H^+$)	Recommended m/z to monitor
1	STVAQLVK(succ)[R]	1111.63452 m/z	556.32090 m/z (z = +2)
2	IGFAEK(succ)VAA[K]	1141.63430 m/z	571.32079 m/z (z = +2)
3	FFK(succ)FS[R]	941.47550 m/z	471.24139 m/z (z = +2)

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

Description: The PTMScan® Control Peptides Succinyl-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: Lysine is subject to a wide array of regulatory post-translational modifications due to its positively charged ε-amino group side chain. The most prevalent of these are ubiquitination and acetylation, which are highly conserved among prokaryotes and eukaryotes (1,2). Acyl group transfer from the metabolic intermediates acetyl-, succinyl-, malonyl-, glutaryl-, butyryl-, propionyl-, and crotonyl-CoA all neutralize lysine's positive charge and confer structural alterations affecting substrate protein function. Lysine acetylation is catalyzed by histone acetyltransferases, HATs, using acetyl-CoA as a cofactor (3,4). Deacylation is mediated by histone deacetylases, HDACs 1-11, and NAD-dependent Sirtuins 1-7. Some sirtuins have little to no deacetylase activity, suggesting that they are better suited for other acyl lysine substrates (5).

Sirt5 is a predominantly mitochondrial desuccinylase and demalonylase (5,6). In the absence of a known succinyltransferase, succinylation is likely driven by the concentration of succinyl-CoA and intracellular pH and is subject to metabolic fluctuations (7,8). Protein succinylation is especially prevalent among mitochondrial metabolic proteins and bacteria, further solidifying the evolutionary link between mitochondria and prokaryotes. It often occurs at lysine residues that are alternatively acetylated or ubiquitinated. More than a thousand lysine succinylation sites were identified on hundreds of proteins, including glutamate dehydrogenase (15 sites), malate dehydrogenase, citrate synthase, carbamoyl phosphate synthase 1, and histone proteins (9).



Extracted ion chromatograms of PTMScan® Control Peptides Succinyl-Lysine added at supplied concentration (1X at 200 fmol) to mouse liver peptides prior to immunoaffinity enrichment using PTMScan® HS Succinyl-Lysine Motif (Succ-K) Kit #60724. Desalted peptides were analyzed on Q Exactive™ mass spectrometer and resolved using a 90 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 a complete listing of recommended complementary 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 Succinyl-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 Succinyl-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 Succinyl-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 Succinyl-Lysine in the LCMS data file.

Background References:

(1) Liu, Z. et al. (2014) *Nucleic Acids Res* 42, D531-6.
(2) Lee, S. (2013) *Toxicol Res* 29, 81-6.
(3) Lin, H. et al. (2012) *ACS Chem Biol* 7, 947-60.
(4) Zhang, Z. et al. (2011) *Nat Chem Biol* 7, 58-63.
(5) Du, J. et al. (2011) *Science* 334, 806-9.
(6) Peng, C. et al. (2011) *Mol Cell Proteomics* 10, M111.012658.
(7) Rardin, M.J. et al. (2013) *Cell Metab* 18, 920-33.
(8) Park, J. et al. (2013) *Mol Cell* 50, 919-30.
(9) Weinert, B.T. et al. (2013) *Cell Rep* 4, 842-51.

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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.