Store at -20°C

PTMScan® Control Peptides Ubiquitin/SUMO

#75964

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	DTGK(gg)TPVEPEVAIH[R]	1772.91651 m/z	443.98458 m/z (z = +4)
2	LIFAGK(gg)QLEDG[R]	1470.79387 m/z	490.93614 m/z (z = +3)
3	TITLEVEPSDTIENVK(gg)A[K]	2109.11651 m/z	703.71035 m/z (z = +3)

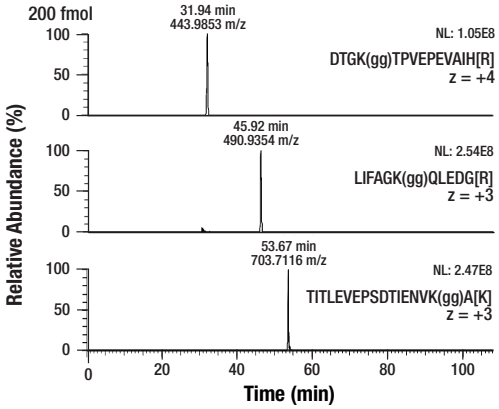
Peptides included in the PTMScan® Control Peptides Ubiquitin/SUMO mix. All peptides are stable-isotope labeled, designated by bracketed R or K, and contain a diglycine remnant designated by parentheses.

Description: The PTMScan® Control Peptides Ubiquitin/SUMO 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: Ubiquitin is a conserved polypeptide unit that plays an important role in the ubiquitin-proteasome pathway. Ubiquitin can be covalently linked to many cellular proteins by the ubiquitination process, which targets proteins for degradation by the 26S proteasome. Three components are involved in the target protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thiolester complex with the activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2, then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH2 of the target protein lysine residue (1-3). The ubiquitin-proteasome pathway has been implicated in a wide range of normal biological processes and in disease-related abnormalities. Several proteins such as IκB, p53, cdc25A, and Bcl-2 have been shown to be targets for the ubiquitin-proteasome process as part of regulation of cell cycle progression, differentiation, cell stress response, and apoptosis (4-7).

Small ubiquitin-related modifier 1, 2, and 3 (SUMO-1, -2, and -3) are members of the ubiquitin-like protein family (8). The covalent attachment of the SUMO-1, -2, or -3 (SUMOylation) to target proteins is analogous to ubiquitination.

Ubiquitin and the individual SUMO family members are all targeted to different proteins with diverse biological functions. Ubiquitin predominantly regulates degradation of its target (8). In contrast, SUMO-1 is conjugated to RanGAP, PML, p53, and IκB-α, regulates nuclear trafficking, forms subnuclear structures, and regulates transcriptional activity and protein stability (9-13). SUMO-2/-3 forms poly-(SUMO) chains, is conjugated to topoisomerase II and APP, regulates chromosomal segregation and cellular responses to environmental stress, and plays a role in the progression of Alzheimer's disease (14-17).



Extracted ion chromatograms of PTMScan® Control Peptides Ubiquitin/SUMO added at supplied concentration (1X at 200 fmol) to mouse liver peptides prior to immunoaffinity enrichment using PTMScan® HS Ubiquitin/SUMO Remnant Motif (K-ε-GG) Kit #59322. 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 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® kit protocol from the Immunoaffinity Purification (IAP) step. Because the optimal amount of PTMScan® Control Peptides Ubiquitin/SUMO 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 Ubiquitin/SUMO 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 Ubiquitin/SUMO 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 Ubiquitin/SUMO in the LCMS data file.

Background References:

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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.