

Store at
-20°C

#34200

1 vial

PTMScan® Control Peptides O-GlcNAc



Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

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

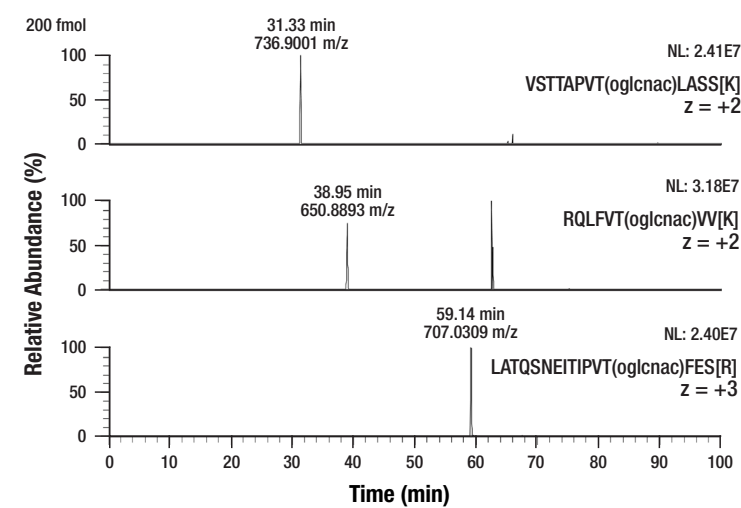
Number	Peptide	Precursor mass (M+H ⁺)	Recommended m/z to monitor
1	VSTTAPVT(oglcnac)LASS[K]	1472.79338 m/z	736.90033 m/z (z = +2)
2	RQLFVT(oglcnac)VV[K]	1300.77146 m/z	650.88937 m/z (z = +2)
3	LATQSNEITIPVT(oglcnac)FES[R]	2119.07928 m/z	707.03128 m/z (z = +3)

Peptides included in the PTMScan® Control Peptides O-GlcNAc mix. All peptides are stable-isotope labeled, designated by bracketed K or R, and contain an N-acetyl-glucosamine group designated by parentheses.

Description: The PTMScan® Control Peptides O-GlcNAc 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: A distinct form of protein glycosylation, beta-linked N-acetyl-glucosamine (GlcNAc) moieties can be added to serine or threonine residues of proteins (1,2). This differs from other forms of glycosylation, as it typically is a single moiety rather than the complex branched sugars that are more commonly studied. It is thought that these modifica-

tions happen in a much more dynamic cycle more reminiscent of phosphorylation modifications. GlcNAc modified proteins are found in the cytoplasm and nucleus and are modulated by means of specific O-GlcNAc transferases (OGT) as well as GlcNAcase activity that can be inhibited using the Thiamet-G (TMG) inhibitor. Mass spectrometry analysis of this modification has been complicated due to the loss of the GlcNAc group during ionization and fragmentation, but methods and technologies such as electron transfer dissociation (ETD) are opening up new avenues to study these modifications. O-GlcNAc could play an important role in many cellular processes, including metabolism, growth, morphogenesis, apoptosis, transcription, and it may play a critical role in cancer (3).



Extracted ion chromatograms of PTMScan® Control Peptides O-GlcNAc added at supplied concentration (1X at 200 fmol) to mouse liver peptides prior to enrichment using PTMScan® O-GlcNAc [GlcNAc-S/T] Motif Kit #95220. 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 O-GlcNAc 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 O-GlcNAc 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 O-GlcNAc 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 O-GlcNAc in the LCMS data file.

Background References:

- (1) Comer, F.I. et al. (2001) *Anal Biochem* 293, 169-77.
- (2) Slawson, C. and Hart, G.W. (2011) *Nat Rev Cancer* 11, 678-84.
- (3) Capotosti, F. et al. (2011) *Cell* 144, 376-88.

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