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-20°C

PTMScan® Control Peptides Phospho-Akt (RXXS*/T*)

#62248

1 vial



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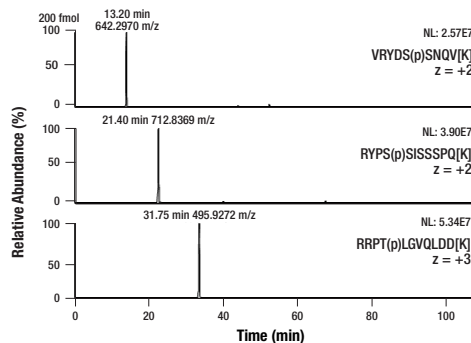
Number	Peptide	Precursor mass (M+H ⁺)	Recommended m/z to monitor
1	VRYDS(p)SNQV[K]	1283.58711 m/z	642.29719 m/z (z = +2)
2	RYPSP(p)SISSSPQ[K]	1424.66609 m/z	712.83668 m/z (z = +2)
3	RRPT(p)LGVQLDD[K]	1485.76647 m/z	495.92701 m/z (z = +3)

Peptides included in the PTMScan® Control Peptides Phospho-Akt (RXXS*/T*) mix. All peptides are stable-isotope labeled, designated by bracketed K, and contain a phosphate group designated by parentheses.

Description: The PTMScan® Control Peptides Phospho-Akt (RXXS*/T*) 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: Akt plays a central role in mediating critical cellular responses, including cell growth and survival, angiogenesis, and transcriptional regulation (1-3). It is a member of an important class of kinases, referred to as Arg-directed kinases or AGC-family kinases, which includes cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), protein kinase C, Akt, p70 S6 kinase, and RSK. These kinases share a substrate specificity characterized by Arg at position -3 relative to the phosphorylated Ser or Thr (4,5). Akt, p70 S6 kinase, and RSK additionally share specificity for Arg at position -5 (recognition sequence RXXXS/T) (6). In a recent phosphoproteomic study (7) co-authored by scientists in the Cell Signaling Technology Site Discovery Group, over 300 downstream substrates for AGC-family kinases recognizing the RXXXS/T motif were identified with PTMScan® Technology using Phospho-Akt substrate antibodies. These Cell Signaling Technology antibodies are powerful tools for investigating the regulation of phosphorylation by Akt and other Arg-directed kinases, as well as for high throughput kinase drug discovery.

In this assay, PTMScan® (RXXS*/T*) Motif Antibody bead conjugates are used to specifically enrich phosphopeptides containing the RXXS*/T* motif (S* = phospho-serine, T* = phospho-threonine).



Extracted ion chromatograms of PTMScan® Control Peptides Phospho-Akt (RXXS*/T*) added at supplied concentration (1X at 200 fmol) to mouse embryo peptides prior to immunoaffinity enrichment using PTMScan® Phospho-Akt Substrate Motif mAb 1 (RXXS*/T*) Kit #5561. 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 Phospho-Akt (RXXS*/T*) 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 Phospho-Akt (RXXS*/T*) 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 Phospho-Akt (RXXS*/T*) 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 Phospho-Akt (RXXS*/T*) in the LCMS data file.

Background References:

- (1) Marte, B.M. and Downward, J. (1997) *Trends Biochem Sci* 22, 355-8.
- (2) Jiang, B.H. et al. (2000) *Proc Natl Acad Sci U S A* 97, 1749-53.
- (3) Scheid, M.P. and Woodgett, J.R. (2000) *Curr Biol* 10, R191-4.
- (4) Montminy, M. (1997) *Annu Rev Biochem* 66, 807-22.
- (5) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol* 200, 62-81.
- (6) Manning, B.D. and Cantley, L.C. (2007) *Cell* 129, 1261-74.
- (7) Moritz, A. et al. (2010) *Sci Signal* 3, ra64.

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