


Store at
-20°C

#80376

PTMScan® Control Peptides
Phospho-Tyrosine

1 vial



Cell Signaling
TECHNOLOGY®

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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	LIEDNEY(p)TA[R]	1313.56486 m/z	657.28607 m/z (z = +2)
2	GY(p)VPAT[K]	936.46816 m/z	468.73772 m/z (z = +2)
3	IGEGTY(p)GVVY[K]	1273.59555 m/z	637.30141 m/z (z = +2)

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

Description: The PTMScan® Control Peptides Phospho-Tyrosine 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: Tyrosine phosphorylation plays a key role in cellular signaling (1). Research studies have shown that in cancer, unregulated tyrosine kinase activity can drive malignancy and tumor formation by generating inappropriate proliferation and survival signals (2). Antibodies specific for phospho-tyrosine (3,4) have been invaluable reagents in these studies. The phospho-tyrosine monoclonal antibodies developed by Cell Signaling Technology are exceptionally sensitive tools for studying tyrosine phosphorylation and monitoring tyrosine kinase activity in high throughput drug discovery.

Extracted ion chromatograms of PTMScan® Control Peptides Phospho-Tyrosine added at supplied concentration (1X at 200 fmol) to HCT 116 human colon cancer cell peptides prior to immunoaffinity enrichment using PTMScan® Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit #8803. 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 Phospho-Tyrosine 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-Tyrosine 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-Tyrosine 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-Tyrosine in the LCMS data file.

Background References:

(1) Schlessinger, J. (2000) *Cell* 103, 211-25.
(2) Blume-Jensen, P. and Hunter, T. (2001) *Nature* 411, 355-65.
(3) Ward, S.G. et al. (1992) *J Biol Chem* 267, 23862-9.
(4) Glenney, J.R. et al. (1988) *J Immunol Methods* 109, 277-85.

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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.