Biotin-Ahx-ubiquitin (pThr12)

Modifying Protein

Alternate Names: Ribosomal Protein S27a, CEP80, UBA80, UBCEP1, UBCEP80, HUBCEP80, RPS27A

Cat. No.	**60-0204-050**	Quantity:	50 µg
Lot. No.	**30363**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

Ubiquitin (Ub) is a highly conserved 76 amino-acid protein found throughout eukaryotic cells. A vast number of cellular processes, including targeted protein degradation, cell cycle progression, DNA repair, protein trafficking, inflammatory response, virus budding, and receptor endocytosis, are regulated by Ub-mediated signalling; where the target protein is tagged by single or multi-monomeric Ub (monomeric Ub attached to multiple sites on the substrate) or a polymeric chain of Ubs (Fushman and Walker, 2010). More recently the demonstration that ubiquitin itself can be modified through phosphorylation by the kinase PTEN Induced putative Kinase1 (PINK1) provides a major breakthrough linking the two most important signalling pathways in cells; phosphorylation and ubiquitylation (Kane *et al.*, 2014; Kazlauskaite *et al.*, 2014; Koyano *et al.*, 2014). Several studies have revealed that PINK1 directly phosphorylates ubiquitin on Ser65 a residue that is also shared by the Parkin Ubl domain (Kane *et al.*, 2014; Kazlauskaite *et al.*, 2014; Koyano *et al.*, 2014). Parkin is activated by Ser65 phosphorylated ubiquitin in a manner which is independent of ubiquitin's ability to be conjugated to lysine residues on target proteins. The mechanism of Parkin priming and activation is thought to occur through a conformational change induced by PINK1 phosphorylation on Ser65 followed by the binding of PINK1 Ser65 phosphorylated ubiquitin on the RING1 domain which optimises the ubiquitylation activ-

Continued on page 2

Physical Characteristics

Species: human

Source: synthetic

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol, 2% DMSO

Molecular Weight: 8.984 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

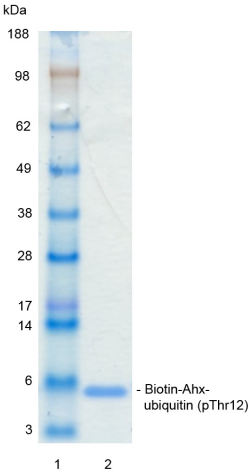
Protein Sequence:

Biotin-Ahx-*M*QIFVKTLTGK(**pT**)ITLEVE
PSDTIENVKAKIQDKEGIPPDQQRLIFAG
KQLEDGRTLSDYNIQKESTLHLVLRLRGG

Tag **(bold text)**: N-terminal Biotin-Ahx (Aminohexanoic acid)
Ubiquitin (regular text): Start ***bold italics*** (amino acid residues 1-76)
Phosphorylated Threonine 12 (**bold** in brackets)
Accession number: P62990.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Biotin-Ahx-ubiquitin (pThr12)



Protein Identification:
Confirmed by mass spectrometry.

UbiQ

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Continued from page 1

ity of Parkin (Kazlauskaite *et al.*, 2014; Koyano *et al.*, 2014). Phospho-ubiquitin may play other roles in regulating Parkin but more generally the identification of phospho-ubiquitin as a second messenger in signalling pathways could reveal the existence of further ubiquitin phosphatases and lead to the discovery of additional kinase and ubiquitin related substrates (Sauve and Gehring, 2014).

Phosphoproteomic studies have identified the presence of several phosphorylated peptides demonstrating homology to proteins of the Ubiquitin Proteasome Pathway (UPP) these include ubiquitin (pThr12 being among those identified), ubiquitin like modifiers and proteins containing ubiquitin binding domains (Bennetzen *et al.*, 2010; Bian *et al.*, 2014; Kettenbach *et al.*, 2011; Sharma *et al.*, 2014; Zhou *et al.*, 2013).

Thus biotinylated versions of phosphorylated ubiquitin that have been identified in such phosphoproteomic studies provide tools for probing the possible roles and functions of these species in signalling pathways (eg in pull down/ capture assays).

Biotin-Ahx-ubiquitin (pThr12) (Cat# 60-0204-050) is a phosphorylated ubiquitin which can be used in experiments alongside the non-phosphorylated control Biotin-Ahx-ubiquitin (synthetic) (Cat# 60-0201-050).

References:

Bennetzen MV, Larsen DH, Bunkenborg J, Bartek J, Lukas J and Andersen JS (2010) Site-specific phosphorylation dynamics of the nuclear proteome during the DNA damage response. *Mol Cell Proteomics*, **9**, 1314-1323.

Bian Y, Song C, Cheng K, Dong M, Wang F, Huang J, *et al.* (2014) An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. *J Proteomics*, **96**, 253-262.

Fushman D and Walker O (2010) Exploring the linkage dependence of polyubiquitin conformations using molecular modeling. *Journal of Molecular Biology*, **395**, 803-814.

Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, *et al.* (2014) PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol*, **205**, 143-153.

Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, Hofmann K, *et al.* (2014) Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J*, **460**, 127-139.

Kettenbach AN, Schweppe DK, Faherty BK, Pechenick D, Pletnev AA and Gerber SA (2011) Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells. *Sci Signal*, **4**, rs5.

Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, *et al.* (2014) Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature*, **510**, 162-166.

Sauve V and Gehring K (2014) Phosphorylated ubiquitin: a new shade of PINK1 in Parkin activation. *Cell Res*, **24**, 1025-6.

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Zhou H, Di Palma S, Preisinger C, Peng M, Polat AN, Heck AJ, *et al.* (2013) Toward a comprehensive characterization of a human cancer cell phosphoproteome. *J Proteome Res*, **12**, 260-271.

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