Biotin-Ahx-ubiquitin (pSer65)

Modifying Protein

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

Cat. No.	**60-0207-050**	Quantity:	50 µg
Lot. No.	**30367**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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). Parkin and PINK1, two major proteins associated with Parkinsons Disease (PD) comprise a mitochondrial quality control pathway that promotes neuronal survival through autophagy of damaged mitochondria in a process known as Mitophagy (Sauve and Gehring, 2014). The accumulation of PINK1 on depolarised or damaged mitochondria leads to the activation and translocation of Parkin to the outer mitochondrial membrane (OMM). Phosphorylation of Parkin by PINK1 at Ser65 located in its Ubl domain markedly increases the E3 ligase activity of Parkin resulting in ubiquitylation of proteins on the OMM, triggering selective Mi-

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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

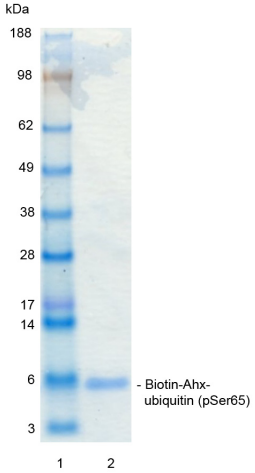
Protein Sequence:

Biotin-Ahx-*M*QIFVKTLTGKTITLEVE
PSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDG
RTLSDYNIQKE(**pS**)TLHLVLRLRGG

Tag **(bold text)**: N-terminal Biotin-Ahx (Aminohexanoic acid)
Ubiquitin (regular text): Start ***bold italics*** (amino acid residues 1-76)
Phosphorylated Serine 65 (**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 (pSer65)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
See page 2.

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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Background

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tophagy (Kondapalli *et al.*, 2012; Spratt *et al.*, 2013; Trempe *et al.*, 2013; Wauer and Komander, 2013).

Several studies have revealed that ubiquitin is also a PINK1 substrate in this pathway where 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 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 of Ser65 on Parkin followed by the binding of PINK1 Ser65 phosphorylated ubiquitin on the RING1 domain which optimises the ubiquitylation activity of Parkin (Kazlauskaite *et al.*, 2014; Koyano *et al.*, 2014). Studies have also identified the presence of at least five phosphorylation sites in Parkin including Ser378, shown to be phosphorylated by Casein kinase1 (CK1) and suggest that further phosphorylation of Parkin may also act to regulate its ubiquitin ligase activity (Yamamoto *et al.*, 2005). Phospho-ubiquitin may play other roles in regulating Parkin but more generally the identification of phosph-ubiquitin as a second messenger in signalling pathways could reveal the existence of further ubiquitin phosphatases and lead to the discovery of additional substrates and signalling functions (Sauve and Gehring, 2014).

Biotin-Ahx-ubiquitin (pSer65) (Cat# 60-0207-050) is a phosphorylated synthetically made ubiquitin which may be used in experiments alongside Ubiquitin (pSer65) (Cat# 60-0202-050) and the non-phosphorylated control Biotin-Ahx-ubiquitin (synthetic) (Cat# 60-0201-050).

References:

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.

Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R, *et al.* (2012) PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol*, **2**, 120080.

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.

Spratt DE, Martinez-Torres RJ, Noh YJ, Mercier P, Manczyk N, Barber KR, *et al.* (2013) A molecular explanation for the recessive nature of parkin-linked Parkinson's disease. *Nat Commun*, **4**, 1983.

Trempe JF, Sauve V, Grenier K, Seirafi M, Tang MY, Menade M, *et al.* (2013) Structure of parkin reveals mechanisms for ubiquitin ligase activation. *Science*, **340**, 1451-1455.

Wauer T and Komander D (2013) Structure of the human Parkin ligase domain in an autoinhibited state. *Embo J*, **32**, 2099-2112.

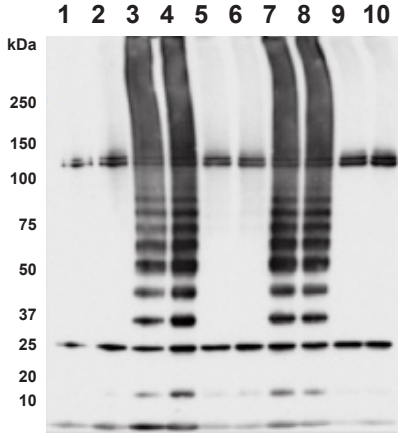
Yamamoto A, Friedlein A, Imai Y, Takahashi R, Kahle PJ and Haass C (2005) Parkin phosphorylation and modulation of its E3 ubiquitin ligase activity. *J Biol Chem*, **280**, 3390-3399.

Quality Assurance

Continued from page 1

Synthetic ubiquitin phosphorylated on Ser65 (ubiquitin (pSer65)) activates Parkin E3 ligase mediated ubiquitylation: Full-length Parkin (2 µg; Cat# 63-0048-025) was incubated at 30˚C with the ubiquitylation assay components Ube1 (0.1 µM; Cat# 61-0001) and Ube2L3 (1 µM; Cat# 62-0042) in the presence of 50 µM ubiquitin (comprising 20 µg of FLAG–ubiquitin mixed with nothing (lanes 1 and 2) or 5 µg of either enzymatically made ubiquitin (pSer65) (lanes 3 and 4), ubiquitin (lanes 5 and 6), synthetically made ubiquitin (pSer65) (Cat# 60-0202-050) (lanes 7 and 8) synthetically made ubiquitin (Cat# 60-0200-050) (lanes 9 and 10). Reactions were terminated after 60 min by the addition of Lithium Dodecyl Sulfate (LDS) loading buffer and products were analysed by Sodium Dodecyl Sulfate (SDS) PAGE followed by immunoblotting. Ubiquitin was detected using an anti-FLAG antibody.

Data generated and kindly provided by A. Kazlauskaite from the Muqit lab at the MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, Scotland, U.K. See Kazlauskaite *et al.* (2014) for details regarding how ubiquitin (pSer65) has been demonstrated to activate the E3 ligase Parkin.



Immunoblot: anti-ubiquitin (Flag)

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