Ubiquitin (synthetic)

Modifying Protein

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

Cat. No.	**60-0200-050**	Quantity:	50 µg
Lot. No.	**30359**	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). This post-translational modification is tightly controlled by an enzymatic cascade involving several enzymes (E1, E2, and E3) and occurs through either an isopeptide bond between the C-terminal Glycyl residue of Ub and the epsilon amino group of a Lysyl residue on a target protein or through a peptide bond between the C-terminal Glycyl residue of Ub and the N-terminal amine on a further Ub. In the former (isopeptide bond-linked) case the substrate protein may either be ubiquitin itself – thus leading to the generation of poly-ubiquitin chains – or another target protein (Fushman *et al.*, 2010). Thus, ubiquitin can be attached to a substrate either as a monomer or as a poly-ubiquitin chain. Further – depending on their linkage type (M1, K6, K11, K27, K29, K33, K48 and K63 linked) – the Ub chains can take different structural forms. Chains containing all eight possible Ub linkages have been found in living cells

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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: 8.645 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

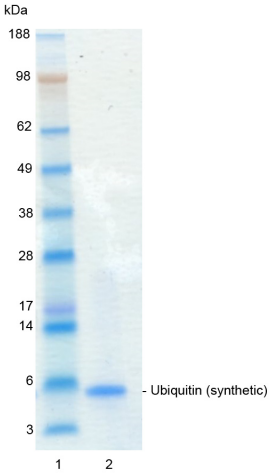
Protein Sequence:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKE
GIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTL
HLVLRLRGG

Ubiquitin (regular text)
Start ***bold italics*** (amino acid residues 1-76)
Accession number: P62990.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Ubiquitin (synthetic)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
See page 2.

UbiQ

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Dundee, Scotland, UK

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Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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

Continued from page 1

and different ubiquitin chain types may encode different biological signals, allowing this single protein to mediate many diverse functions (Komander 2009; Weeks *et al.*, 2009; Walczak *et al.*, 2012). The functionality of Ub chains is most commonly associated with their attachment to substrate proteins but there is also evidence that they may also play a role in cellular signalling as free chains (Braten *et al.*, 2012).

Ubiquitin also acts as a second messenger in the proteasomal degradation pathway. In a dual mechanism, the kinase PTEN Induced putative Kinase1 (PINK1) phosphorylates Ser65 on ubiquitin and Ser65 in the Ubl domain of the E3 ligase Parkin. Phosphorylation of Parkin at Ser65 results in the opening of the Parkin structure to allow the binding of Ser65 phosphorylated ubiquitin thus activating the E3 ligase (Kane *et al.*, 2014; 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 substrates and signalling functions (Sauve and Gehring, 2014).

Ubiquitin (synthetic) (Cat# 60-0200-050) is a non-phosphorylated synthetically made ubiquitin which may be used in experiments as a control alongside Ubiquitin (pSer65) (Cat# 60-0202-050) and other varieties of modified synthetic ubiquitin.

References:

Braten O, Shabek N, Kravtsova-Ivantsiv Y, Ciechanover A (2012) Generation of free ubiquitin chains is upregulated in stress, and facilitated by the HECT domain ubiquitin ligases UFD4 and HUL5. *Biochem J* **444**, 611-617.

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.

Komander D (2009) The emerging complexity of protein ubiquitination. *Biochem Soc Trans* **37**, 937-953.

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.

Walczak H, Iwai K, Dikic I (2012) Generation and physiological roles of linear ubiquitin chains. *BMC Biol* **10**, 23.

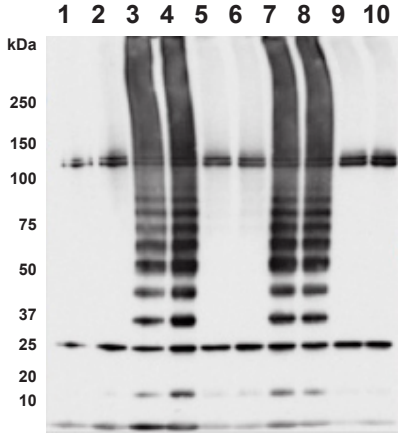
Weeks SD, Grasty KC, Hernandez-Cuebas L, Loll PJ (2009) Crystal structures of Lys-63-linked tri- and di-ubiquitin reveal a highly extended chain architecture. *Proteins* **77**, 753-759.

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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