Biotinylated Ubiquitin

Modifier

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

Cat. No.	**60-0121-020**	Quantity:	20 µg
Lot. No.	**30219**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

Biotinylated ubiquitin is a powerful research tool for detecting ubiquitylation *in vitro*. The biotinyl moiety allows for the use of high affinity streptavidin detection reagents and allows for the isolation of ubiquitin conjugated substrates by avidin/streptavidin purification methods.

Ubiquitin is a highly conserved protein that plays a key role in the ubiquitylation pathway. Ubiquitin is found only in eukaryotic organisms throughout which it shows strong sequence conservation (Wilkinson, 1995). The ubiquitin protein is present in all cell types and found either in free form or conjugated to proteins through a covalent bond between its C-terminal glycine and the ε-amino group of lysine residues or the α-amino group of an N-terminal methionine; a process known as ubiquitination or ubiquitylation. Ubiquitylation is an essential cellular process affected by a multi-enzyme cascade involving three classes of enzyme known as activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). E1 activates ubiquitin in an ATP-dependent manner resulting in the formation of a thioester linkage between the carboxy terminus of ubiquitin and the E1 enzyme. Sequential, transient thioester bonds are then generated between the carboxy terminus of ubiquitin and specific cysteines of the E2 and – in some instances – of the E3 enzymes (Bonifacino and Weissman, 1998). Ultimately, an isopeptide bond is formed between the glycine carboxy terminus of ubiquitin and an ε-amino group of a lysine

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Physical Characteristics

Species: bovine

Source: erythrocytes

Quantity: 20 µg

Concentration: 1.0 mg/ml

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

Molecular Weight: ~9.8 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

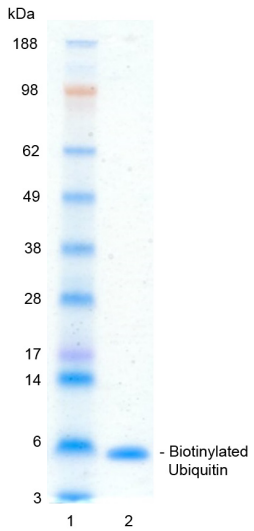
MQIFVK**(biotinyl)**TLTGKTITLEVEPSDTIE
NVKAKIQDKEGIPPDQQRLIFAGK**(biotinyl)**
QLEDGRTLSDYNIQKESTLHLVLRLRGG

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

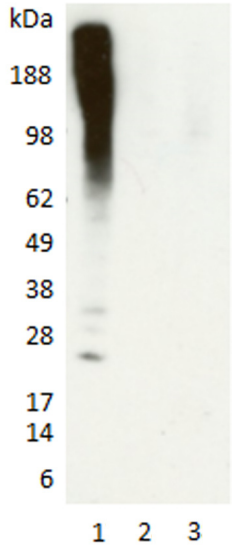
Mass spectrometry confirmed that the predominant sites of biotinylation are on Lys6 and Lys48 although other Lys residues may be biotinylated to a lesser degree.

Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: Activity of the biotinylated ubiquitin was validated through its ability to be incorporated into polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D3 (UbcH5c) and the E3 ligase His-IDOL. Incubation of biotinylated ubiquitin for 60 minutes at 30˚C in the presence of His-IDOL, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or His-IDOL Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an HRP-linked streptavidin conjugate and these were observed only in the presence of both ATP and His-IDOL. The reaction (Lane 1) contains a number of ubiquitylated and biotinylated species.

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Lot-specific COA version tracker: v1.0.0

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residue on a target protein (mono-ubiquitylation) or on another ubiquitin resulting in the generation of chains of ubiquitin (poly-ubiquitylation) which may be Lys-6, Lys-11, Lys-27, Lys-29, Lys-33, Lys-48 or Lys-63 linked (Komander, 2009). Ubiquitin chains may also be linear in nature, formed via the conjugation of the activated glycine residue of one ubiquitin moiety to the α-amino group at the N-terminus of another ubiquitin. Specific ubiquitin chain types adopt distinct conformations which are likely to be important in respect of their functions. Although some functionalities have been determined for certain chain types, the roles of many of these structures remain to be fully elucidated (Komander, 2009). In respect of Lys-48 and Lys-63 chain types, some key roles have been determined: Lys-48 linked chains direct substrates towards 26S proteasome mediated degradation (Verma *et al.*, 2004), whereas roles for Lys-63 linked chains include activation of the NF-κB pathway and mediation of steps of the DNA repair pathway (DiFiglia *et al.*, 1997; Rahighi *et al.*, 2009; Tokunaga *et al.*, 2009). Interestingly, proteins constituting many types of pathological inclusion bodies may be poly-ubiquitylated, however these may be resistant to degradation. For example poly-ubiquitylated huntingtin accumulates at neuronal intranuclear inclusions (NIIs) and dystrophic neuritis in the striatum and cortex of patients affected by Huntington's disease (DiFiglia *et al.*, 1997).

References:

Bonifacino JS, Weissman AM (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu Rev *Cell Dev Biol* **14**, 19-57.

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Rahighi S, Ikeda F *et al.* (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* **136**, 1098-109.

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Wilkinson KD (1995) Roles of ubiquitinylation in proteolysis and cellular regulation. *Annu Rev Nutr* **15**, 161-89.

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Lot-specific COA version tracker: v1.0.0