Ubiquitin

Modifying Protein

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

Cat. No.	60-0001-025	Quantity:	25 mg
Lot. No.	30560	Storage:	4˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

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 e-amino group of lysine residues; 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 e-amino group of a lysine 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 a-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

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Physical Characteristics

Species: bovine

Source: erythrocytes

Quantity: 25 mg

Formulation: Lyophilized (from protein dissolved in H_2O)

Reconstitution: Reconstitute the lyophilized material as required (add 500 µl H_2O for a 50 mg/ml stock). After adding the H_2O, re-cap the vial and mix by vortexing thoroughly then place on a rolling platform. Allow the vial to sit at room temperature with gentle mixing for at least 15 minutes. Vortex thoroughly before use.

Molecular Weight: ~8.6 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

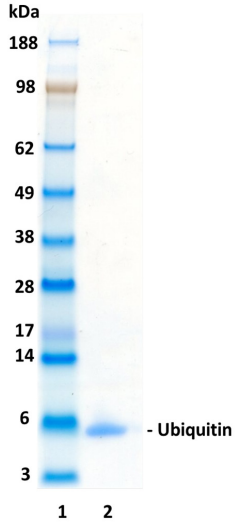
Stability/Storage: 1 year lyophilized at 4˚C; 6 months rehydrated at -20˚C; aliquot as required

Protein Sequence:
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQD
KEGIPPDQQRLIFAGKQLEDGRTLSDY
NIQKESTLHLVLRLRGG

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



E2-Ubiquitin Thioester Loading Assay:
The activity of ubiquitin was validated by loading ubiquitin onto the active cysteine of the UBE2N E2 enzyme via a transthiolation reaction. Incubation of ubiquitin, UBE1 and UBE2N enzymes in the presence of ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the ubiquitin/UBE2N thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0

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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-kB 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 neurites 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.

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277, 1990-3.

Komander D (2009) The emerging complexity of protein ubiquitination. Biochem Soc Trans 37, 937-53.

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.

Tokunaga F, Sakata S, et al. (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. Nat Cell Biol 11, 123-32.

Verma R, Oania R, Graumann J, Deshaies RJ (2004) Multiubiquitin chain receptors define a layer of substrate selectivity in the ubiquitin-proteasome system. Cell 118, 99-110.

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

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