Di-ubiquitin (K6-linked) [untagged]

Ubiquitin/Ubiquitin-Like Protein Dimer

Cat. No. **60-0101-010**
Lot. No. **30082**

Quantity: 10 µg
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 *et al.*, 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 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).

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Physical Characteristics

Protein Sequence:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

K6

Species: human

Source: synthetic/chemical ligation

Quantity: 10 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM $NaCl_2$, 2 mM DTT, 10% Glycerol

Molecular Weight: 17.1 kDa

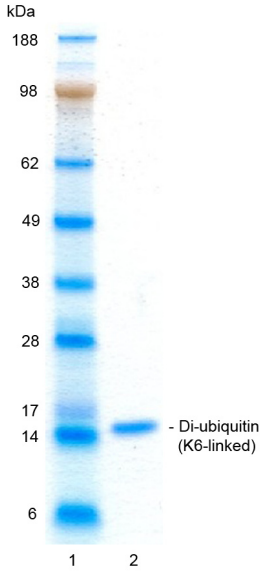
Purity: >98% by InstantBlue™ SDS-PAGE

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

Accession Number: P62987

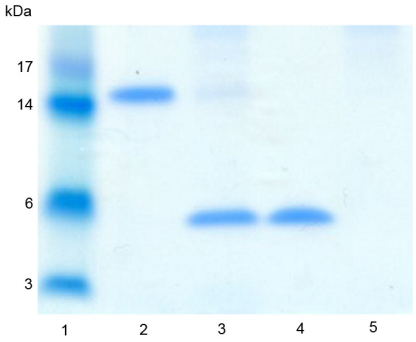
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Di-ubiquitin (K6-linked)



Purity of the linkage type:
The linkage type (K6) was confirmed by tandem mass spectrometry.

Di-ubiquitin cleavage assay:
The capacity of the di-ubiquitin substrate to be cleaved was tested using a promiscuous – with respect to ubiquitin linkage specificity – deubiquitylase (GST-USP2). Incubation of the di-ubiquitin for 1 hour at 37˚C was compared either in the absence (Lane 2) or presence (Lane 3) of GST-USP2. The reaction products were compared alongside two control samples containing either mono-ubiquitin (Lane 4) or GST-USP2 (Lane 5) only. Cleavage of the di-ubiquitin and generation of mono-ubiquitin was determined by running reactions on a 4-12% SDS-PAGE gel and staining with InstantBlue™ (Lane 1; molecular weight markers).



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Lot-specific COA version tracker: v1.0.0

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A mass spectrometry-based study found that K6 linkages account for 11% of all yeast ubiquitin-ubiquitin linkages. The relative abundance of the other linkages were K11 (28%), K27 (9%), K29 (3%), K33 (4%), K48 (29%) and K63 (16%) (Xu *et al.*, 2009). A well characterised heterodimeric RING E3 ligase complex consisting of the breast cancer-susceptibility proteins BRCA1 and BARD1 was shown to mediate K6-linked polyubiquitylation. However, the physiological roles of this chain type are currently unclear. Individuals who carry mutations in the BRCA1 gene are predisposed to early-onset breast and ovarian cancer. BRCA1/BARD1 are localized at sites of DNA damage, through binding of their adaptor protein RAP80 to K6- and K63-linked ubiquitin chains. Hence, both chain types may be involved in DNA Repair (Wu-Baer *et al.*, 2003).

The importance of the K6 residue *per se* has been investigated and it has been found that expression of a K6W mutant version of ubiquitin inhibits ubiquitin-dependent substrate proteolysis in mammalian cells. Furthermore Lys6-modified ubiquitin in cell-free assays significantly and specifically inhibited ATP-dependent proteolysis (Shang *et al.*, 2005). It is speculated that the mechanism underlying the above phenomena may be related to the attenuated avidity of the interaction between K6-modified ubiquitin conjugates and the 26S proteasome. Furthermore it has been found that of the seven Lys residues in ubiquitin, Lys6 is the most readily modifiable with sulfo-NHS-biotin and other chemical reagents. Thus one can speculate that *in vivo* modifications of ubiquitin Lys6 are not only plausible but may interfere with proteasomal degradation and result in the accumulation of ubiquitin conjugates. Perhaps explaining why ubiquitin conjugates are accumulated in response to oxidative stress and upon aging (Shang *et al.*, 2005).

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, Walker O (2010) Exploring the linkage dependence of polyubiquitin conformations using molecular modeling. *J Mol Biol* **395**, 803-814.

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

Shang F, Deng G, Liu Q, Guo W, Haas AL, *et al.*, (2005) Lys6-modified ubiquitin inhibits ubiquitin-dependent protein degradation. *J Biol Chem* **280**, 20365-20374.

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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.

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Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, *et al.*, (2009) Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* **137**, 133-145.

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