Di-ubiquitin (K29-linked) [untagged]

Ubiquitin/Ubiquitin-Like Protein Dimer

Cat. No. **60-0104-010**
Lot. No. **30085**

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

K29

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

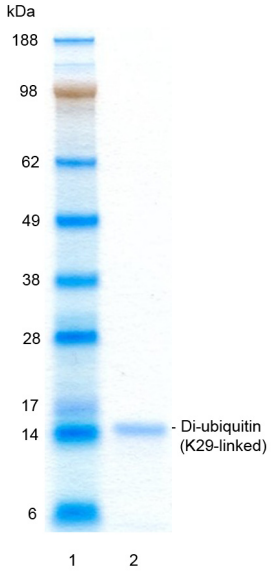
Purity: >98% by InstantBlue™ SDS-PAGE

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

Accession Number: P62987

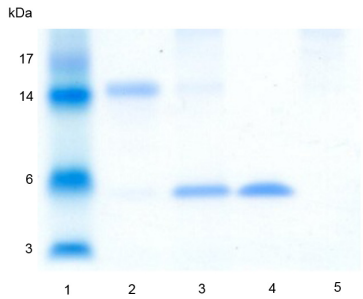
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Di-ubiquitin (K29-linked)



Purity of the linkage type:
The linkage type (K29) was confirmed by tandem mass spectrometry. A small (~10%) amount of K27 linkage was also detected in the analysis of the sample mass spectrometry data.

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 K29 linkages account for just 3% of all yeast ubiquitin-ubiquitin linkages. The relative abundance of the other linkages were K6 (11%), K11 (28%), K27 (9%), K33 (4%), K48 (29%) and K63 (16%) (Xu *et al.*, 2009). A recent study has shown that an E3 ubiquitin ligase identified by differential display (EDD; E3 ubiquitin ligase identified by Differential Display) enhanced nuclear accumulation of both GSK-3β and β-catenin. EDD ubiquitylates β-catenin with Lys29- and/or Lys11-linked ubiquitin chains, leading to enhanced stability of β-catenin thus suggesting a potentiating role for ubiquitylation by EDD in the Wnt signalling pathway and cancer development (Hay-Koren *et al.*, 2011). The E3 ligases Deltex (DTX) and AIP4 are known to be antagonistically involved in the Notch signalling pathway. AIP4 targets DTX for lysosomal degradation and generates polyubiquitin chains *in vivo* that are mainly K29-conjugated (including on DTX), indicating a link between this chain type and lysosomal degradation (Chastagner *et al.*, 2006).

References:

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Fushman D, Walker O (2010) Exploring the linkage dependence of polyubiquitin conformations using molecular modeling. *Journal of Molecular Biology* **395**, 803-814.

Hay-Koren A, Caspi M, Zilberberg A, Rosin-Arbesfeld R (2011) The EDD E3 ubiquitin ligase ubiquitinates and up-regulates beta-catenin. *Molecular Biology of the Cell* **22**, 399-411.

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

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.

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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Lot-specific COA version tracker: v1.0.0