# Di-ubiquitin (K33-linked) [untagged]

Ubiquitin/Ubiquitin-Like Protein Dimer

**Cat. No.** **60-0105-010**
**Lot. No.** **30086**

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).

*Continued on page 2*

## Physical Characteristics

**Protein Sequence:**

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

K33

**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

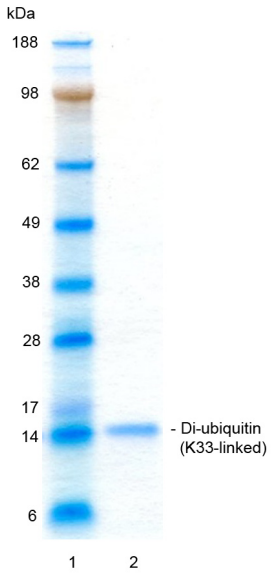
**Purity:** >98% by InstantBlue™ SDS-PAGE

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

**Accession Number:** P62987

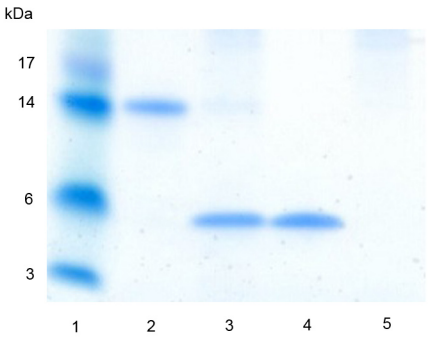
## Quality Assurance

**Purity:**
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Di-ubiquitin (K33-linked)




**Purity of the linkage type:**
The linkage type (K33) 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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## Background

*Continued from page 1*

A mass spectrometry-based study found that K33 linkages account for just 4% of all yeast ubiquitin-ubiquitin linkages. The relative abundance of the other linkages were K6 (11%), K11 (28%), K27 (9%), K29 (3%), K48 (29%) and K63 (16%) (Xu *et al.*, 2009). AMPK (AMP-activated protein kinase)-related kinases, NUAK1 and MARK4 have been shown to be polyubiquitylated *in vivo* and interact with the deubiquitylase enzyme USP9x. These AMPK-related kinases regulate cell polarity as well as proliferation and are known to be activated by the LKB1-tumour suppressor kinase. NUAK1 and MARK4 have been shown to be conjugated to K29 and/or K33 ubiquitin chains rather than the more common Lys48/Lys63. Ubiquitylation of NUAK1 and MARK4 does not control their stability, but instead inhibits their phosphorylation and activation by LKB1 (Al-Hakim *et al.*, 2008). A recent study characterizes K33-linked polyubiquitylation of T cell receptor-zeta (TCR-ζ) and indicates the important role of unconventional cell surface receptor ubiquitylation in regulating T cell activation in a proteolysis-independent manner (Huang *et al.*, 2010).

**References:**


Al-Hakim AK, Zagorska A, Chapman L, Deak M, Peggie M, *et al.*, (2008) Control of AMPK-related kinases by USP9X and atypical Lys(29)/Lys(33)-linked polyubiquitin chains. *Biochem J* **411**, 249-260.

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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.

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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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