Di-ubiquitin (K63-linked) [untagged]

Ubiquitin/Ubiquitin-Like Protein Dimer

Cat. No. **60-0107-050**
Lot. No. **30088**

Quantity: 50 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

K63

Species: human

Source: enzyme catalysed

Quantity: 50 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

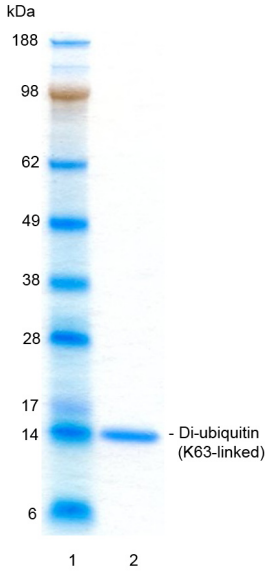
Purity: >98% by InstantBlue™ SDS-PAGE

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

Accession Number: P62987

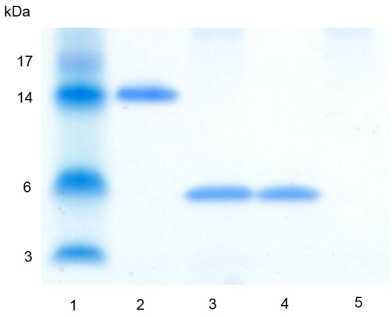
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Di-ubiquitin (K63-linked)



Purity of the linkage type:
The linkage type (K63) 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 K63 linkages account for 16% of all yeast ubiquitin-ubiquitin linkages. The relative abundance of the other linkages were K6 (11%), K11 (28%), K27 (9%), K29 (3%), K33 (4%) and K48 (29%) (Xu *et al.*, 2009). Ubiquitin chains form distinct structures based on their linkage: K63-linked chains adopt an extended conformation with little interaction between adjacent ubiquitins. In contrast, K11- and K48-linked chains form compact, globular structures with significant ubiquitin-ubiquitin contact (Schaefer *et al.*, 2011). Polyubiquitin chains of different linkage types have distinct biological functions. The best understood among these are the K48-linked ubiquitin chains, which target proteins for degradation by the 26S proteasome. K63-linked ubiquitin chains, on the other hand, play non-degradative roles in different signalling pathways, notably NFκB transcription activation and the DNA damage response (Datta *et al.*, 2009). Although this type of ubiquitin chain exerts its effects at different stages of the NFκB pathway, its key regulatory functions are in influencing the assembly and stability of IKK-activating complexes and in kinase activation (Schmukle *et al.*, 2012). NEMO (NFκB Essential Modifier) is the prototypic member of a family of proteins that interact with K63-linked and linear polyubiquitin chains (Ordureau *et al.*, 2008; Nanda *et al.*, 2011). It is an integral component of the canonical IκB kinase (IKK) complex and is essential for the activation of IKKα and IKKβ, the protein kinase components of the complex. NEMO is a powerful reagent for capturing the K63-linked and linear polyubiquitin chains and their binding partners present in cell extracts.

References:

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Schaefer JB, Morgan DO (2011) Protein-linked ubiquitin chain structure restricts activity of deubiquitinating enzymes. *J Biol Chem* **286**, 45186-45196.

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