Di-ubiquitin (linear) [GST-cleaved]

Ubiquitin/Ubiquitin-Like Protein Dimer

Cat. No. **60-0115-010**
Lot. No. **30108**

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

Species: human

Source: *E. coli*

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: 17.7 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

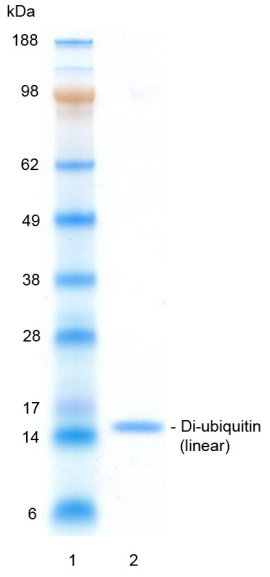
GPLGSAG**M**QIFVKTLTGKTITLEVEPSDTIEN
VKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLS
DYNIQKESTLHLVLRLRGG┐
└MQIFVKTLTGKTITLEVEPSDTIENVKAKI
QDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQ
KESTLHLVLRLRGG

The residues underlined remain after cleavage and removal of the purification tag.
Linear di-ubiquitin (regular text): Start ***bold italics.***

Accession Number: P62987

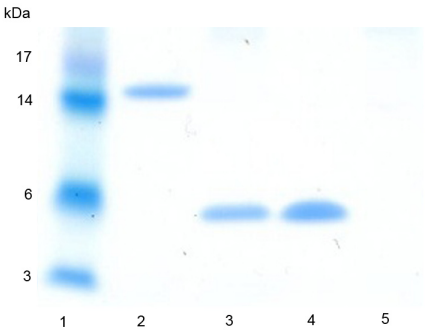
Quality Assurance

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



Purity of the linkage type:
The linkage type (linear) 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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www.ubiquigent.com
Dundee, Scotland, UK

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US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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Lot-specific COA version tracker: v1.0.0

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In contrast to Lys-48 di-ubiquitin both Lys-63 and linear di-ubiquitin adopt open conformations with no contact between the ubiquitin molecules (Komander *et al.*, 2009). Reflecting these different structures alternative ubiquitin chain types can display varying specificities for ubiquitin binding domains. In fact the ubiquitin-chain binding protein NEMO (Nuclear factor-κB (NF-κB) essential modulator) shows preference for linear ubiquitin chains even over the similarly structured Lys-63 chains (Kensche *et al.*, 2012); NEMO being part of the IKK-complex along with IKKα and IKKβ. The binding of the adaptor protein NEMO to ubiquitin chains appears to be critical to the linking of upstream ubiquitin signals with the activation of this complex and subsequently the NF-κB pathway. The LUBAC complex has been identified as an E3 ligase consisting of three subunits – SHARPIN, HOIL-IL and HOIP – which linearly ubiquitylates NEMO leading to activation of the IKK kinases (Tokunaga *et al.*, 2009; Gerlach *et al.*, 2011; Tokunaga *et al.*, 2012).

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. *Journal of Molecular Biology* **395**, 803-814.

Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, *et al.*, (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591-596.

Kensche T, Tokunaga F, Ikeda F, Goto E, Iwai K, *et al.*, (2012) Analysis of NF-kappaB essential modulator (NEMO) binding to linear and lysine-linked ubiquitin chains and its role in the activation of NF-kappaB. *J Biol Chem* **287**, 23626-23634

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

Komander D, Reyes-Turcu F, Licchesi JD, Odenwaelder P, Wilkinson KD, *et al.*, (2009) Molecular discrimination of structurally equivalent Lys 63-linked and linear polyubiquitin chains. *EMBO Rep* **10**, 466-473.

Tokunaga F, Iwai K (2012) LUBAC, a novel ubiquitin ligase for linear ubiquitination, is crucial for inflammation and immune responses. *Microbes Infect* **14**, 563-572.

Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, *et al.*, (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* **11**, 123-132.

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.

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Dundee, Scotland, UK

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UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
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