

Di-ubiquitin (K48-linked) [untagged]

Ubiquitin/Ubiquitin-Like Protein Dimer



Cat. No. 60-0106-010

Lot. No. 30087

Quantity: 10 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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 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:

```
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQORLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG  
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQORLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG  
K48
```

Species: human

Molecular Weight: 17.1 kDa

Source: enzyme catalysed

Purity: >98% by InstantBlue™ SDS-PAGE

Quantity: 10 µg

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

Concentration: 0.5 mg/ml

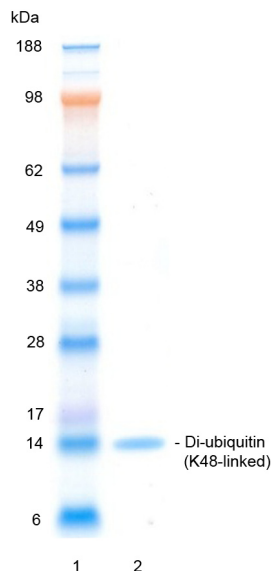
Accession Number: P62987

Formulation: 50 mM HEPES pH 7.5, 150 mM NaCl₂, 2 mM DTT, 10% Glycerol

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Di-ubiquitin (K48-linked)



Purity of the linkage type:

The linkage type (K48) 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

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A mass spectrometry-based study found that K48 linkages account for the largest percentage (29%) 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 K63 (16%) (Xu *et al.*, 2009). The attachment of K48-linked polyubiquitin chains to proteins is a universal signal for degradation by the proteasome. 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). Lys48-linked ubiquitin tetramers are the minimal recognition motif for the proteasome and a K48-ubiquitinated substrate will be degraded within minutes in cells (Thrower *et al.*, 2000).

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.

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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.

Thrower JS, Hoffman L, Rechsteiner M, Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. *The EMBO Journal* **19**, 94-102.

Walczak H, Iwai K, Dikic I (2012) Generation and physiological roles of linear ubiquitin chains. *BMC Biol* **10**, 23.

Weeks SD, Grasty KC, Hernandez-Cuevas 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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