MATERIAL DATA SHEET

Recombinant Human Di−Ubiquitin/Ub2 Non-hydrolyzable (K63)
Cat. # UCN-300

With a predicted molecular weight of 17 kDa, Di-Ubiquitin is composed of two Ubiquitin monomers that are covalently linked through an isopeptide bond, which typically form between a lysine residue of one Ubiquitin molecule and the C-terminal glycine residue of another Ubiquitin molecule (1). Each human Ubiquitin monomer is 76 amino acids (aa) in length and shares 96% and 100% aa identity with yeast and mouse Ubiquitin, respectively (2). Ubiquitin has seven lysine residues that can participate in the formation of poly-Ubiquitin chains. The specific lysine residue used in Ubiquitin conjugation is thought to determine the function of poly-ubiquitination in cellular processes such as protein degradation, signaling, and trafficking (3-8).

Linkage specific, non-hydrolyzable di-Ubiquitin is resistant to the activity of deubiquitinating enzymes (DUB's) that cleave the isopeptide linkage between adjacent Ubiquitin molecules. It can be used to investigate binding interactions between di-Ubiquitin and proteins that contain elements such as Ubiquitin-associated domains (UBAs) or Ubiquitin-interacting motifs (UIMs). This product may also be useful in exploring the role of unanchored poly-Ubiquitin chains in some signaling pathways.

Product Information	
Quantity:	100 µg
MW:	17 kDa
Source:	*E. coli*-derived Accession # P0CG47 Each Ubiquitin contains a Pro substitution at position 73.
Stock:	Lyophilized from a solution in deionized water.
Solubility:	Reconstitute at 5 mg/ml in aqueous buffer.
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

Use & Storage

Use: Ubiquitin chains vary in length, linkage, and function. K63-linked Non-hydrolyzable Di-Ubiquitin Chains (Ub2) may be useful for investigating Ubiquitin-binding proteins and exploring the role of unanchored Ubiquitin chains in signaling pathways. Reaction conditions will need to be optimized for each specific application. IMPORTANT: Heating this product in SDS-PAGE buffer or terminating reactions containing this product with heated SDS-PAGE buffer could lead to unexpected, high apparent molecular weight banding or smearing on gels that is not representative of product purity. For optimal results, we recommend incubation in SDS-PAGE buffer + DTT at <40 °C for 20 minutes prior to gel electrophoresis.

Storage: **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

Literature

References:

1. Scheffner, M. *et al*. (1995) Nature **373**:81.
2. Sharp, P.M. & W.-H. Li (1987) Trends Ecol. Evol. **2**:328.
3. Behrends, C. & J.W. Harper (2011) Nat. Struct. Mol. Biol. **18**:520.
4. Greene, W. *et al*. (2012) PLoS Pathog. **8**:e1002703.
5. Henry, A.G. *et al*. (2012) Dev. Cell **23**:519.
6. Tong, X. *et al*. (2012) J. Biol. Chem. **287**:25280.
7. Wei, W. *et al*. (2004) Nature **428**:194.
8. Zhang, J. *et al*. (2012) J. Biol. Chem. **287**:28646.

For research use only. Not for use in humans.