

# **MATERIAL DATA SHEET**

## **Recombinant Human His6 Ubiquitin**

### Cat. # U-530

Ubiquitin is a 76 amino acid (aa) protein that is ubiquitously expressed in all eukaryotic organisms. Ubiquitin is highly conserved with 96% aa sequence identity shared between human and yeast Ubiquitin, and 100% aa sequence identity shared between human and mouse Ubiquitin (1). In mammals, four Ubiquitin genes encode for two Ubiquitin-ribosomal fusion proteins and two poly-Ubiquitin proteins. Cleavage of the Ubiquitin precursors by deubiquitinating enzymes gives rise to identical Ubiquitin monomers each with a predicted molecular weight of 8.6 kDa. Conjugation of Ubiquitin to target proteins involves the formation of an isopeptide bond between the C-terminal glycine residue of Ubiquitin and a lysine residue in the target protein. This process of conjugation, referred to as ubiquitination or ubiquitylation, is a multi-step process that requires three enzymes: a Ubiquitin-activating (E1) enzyme, a Ubiquitin-conjugating (E2) enzyme, and a Ubiquitin ligase (E3). Ubiquitination is classically recognized as a mechanism to target proteins for degradation and as a result, Ubiquitin was originally named ATP-dependent Proteolysis Factor 1 (APF-1) (2,3). In addition to protein degradation, ubiquitination has been shown to mediate a variety of biological processes such as signal transduction, endocytosis, and post-endocytic sorting (4-7).

Functional N-terminal His-tagged Ubiquitin which allows for metal chelate affinity purification of ubiquitinated proteins.

Product Information	
Quantity:	2 mg
MW:	9.6 kDa
Source:	<i>E. coli</i> -derived human Ubiquitin protein Contains an N-terminal 6-His tag Accession # P0CG47
Stock:	2.4 mg/ml (250 $\mu M)$ in 10 mM HEPES pH 7.5
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

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#### Use & Storage

Use:	Recombinant Human His6-Ubiquitin can be conjugated to substrate proteins via the subsequent actions of a Ubiquitin-activating (E1) enzyme, a Ubiquitin-conjugating (E2) enzyme, and a Ubiquitin ligase (E3). Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human His6-Ubiquitin concentration of 0.2-1 mM.
Storage:	<ul> <li>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</li> <li>24 months from date of receipt, -20 to -70 °C as supplied.</li> <li>3 months, -20 to -70 °C under sterile conditions after opening.</li> </ul>

### Literature

### **References:**

- 1. Sharp, P.M. & W.-H. Li. (1987) Trends Ecol. Evol. 2:328.
- 2. Ciechanover, A. et al. (1980) Proc. Natl. Acad. Sci. USA 77:1365.
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- 4. Greene, W. et al. (2012) PLoS Pathog. 8:e1002703.
- 5. Tong, X. et al. (2012) J. Biol. Chem. 287:25280.
- 6. Wei, W. et al. (2004) Nature 428:194.
- 7. Wertz, I.E. et al. (2004) Nature 430:694.

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