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MATERIAL DATA SHEET

Recombinant Human Ubiquitin Biotin
Cat. # UB-570

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).

Ubiquitin modified with Biotin via primary amine coupling results in multiple biotinylated Ubiquitin species modified at the N-terminus, as well as lysine residues. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-Ubiquitin chains. Biotinylated Ubiquitin can be detected using avidin-linked reagents.

Product Information	
Quantity:	100 µg
MW:	8.6 kDa (unlabeled), extent of biotinylation varies by lot
Source:	*E. coli*-derived human Ubiquitin protein Accession # P0CG47
Stock:	X mg/ml in 10 mM HEPES pH 7.5
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

Use & Storage

Use: Biotinylated Recombinant Human 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 using Biotinylated Recombinant Human Ubiquitin in conjunction with native Ubiquitin at a combined concentration of 100-500 µM with a 1:1 to 1:5 ratio of Biotinylated Recombinant Human Ubiquitin:native Ubiquitin. The resulting poly-Ubiquitin chains can be visualized/quantitated with avidin-linked detection reagents

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

- 24 months from date of receipt, -20 to -70 °C as supplied.
- 12 months, -20 to -70 °C under sterile conditions after opening.

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.
3. Hershko, A. et al. (1980) Proc. Natl. Acad. Sci. USA **77**:1783.
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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