

MATERIAL DATA SHEET**Biotinylated Ubiquitin, *human recombinant*****Cat. # U-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. 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 Enzyme (E1), 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). 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. Highly purified ubiquitin processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and *in vitro* reactions.

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 partially limit the ability of this reagent to propagate polyubiquitin chains. Biotinylated ubiquitin can be detected using avidin-linked reagents.

Product Information

Quantity:	100 µg, lyophilized
MW:	8.6 kDa a (unlabeled), extent of biotinylation varies by lot
Solubility:	Reconstitute at 5 mg/ml in aqueous buffer
Purity:	> 95% by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie Blue stain

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Use & Storage

Use:	Biotinylated Recombinant Human ubiquitin can be conjugated to substrate proteins via the subsequent actions of a Ubiquitin-Activating Enzyme (E1), a Ubiquitin-Conjugating Enzyme (E2), 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 μ M with a 1:1 to 1:5 ratio of biotinylated ubiquitin:native ubiquitin. The resulting poly-Ubiquitin chains can be visualized or captured with avidin-linked reagents.
Storage:	Lyophilized powder at 4°C. Solubilized stock solution at -20°C. Avoid multiple freeze/thaw cycles.

Literature

References:	Ciechanover, A. <i>et al.</i> (1980) <u>Proc. Natl. Acad. Sci. USA</u> 77 : 1365
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