

MATERIAL DATA SHEET

Recombinant Human His6 Ubiquitin Mutant G76A

Cat. # UM-HG76A

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).

Mature Ubiquitin has a highly conserved C-terminal diglycine motif which is crucial for activity and recognition with conjugation and deconjugation enzyme components. The replacement the last glycine residue with alanine results in a Ubiquitin that supports Ubiquitin-activating (E1) enzyme Ubiquitin thioester formation and downstream conjugation reactions (transfer to E2, E3) but at a rate ~20% compared to wild type Ubiquitin. This mutant however, inhibits deconjugation and prevents the removal of Ubiquitin from modified protein substrates by deubiquitinating enzyme (DUBs). Since this Ubiquitin becomes irreversibly conjugated to protein, it shifts the equilibrium between the bound and unbound form in the direction of conjugation, at the expense of the free form. This protein contains an N-terminal His6-tag.

Product Information

Quantity:	100 µg
Source:	<i>E. coli</i> -derived Contains an N-terminal 6-His tag Accession # P62988
Stock:	Lyophilized from a solution in deionized water
Solubility:	Reconstitute at 10 mg/mL in an aqueous solution.
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

Use & Storage

Use: 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). Recombinant Human His6-Ubiquitin Mutant G76A is able to form chains that are resistant to deubiquitinating enzyme activity. Reaction conditions will need to be optimized for each specific application.

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

- 12 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. Sharp, P.M. & W.-H. Li. (1987) Trends Ecol. Evol. **2**:328.
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3. Herskho, 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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