

## MATERIAL DATA SHEET

### Recombinant Human Ubiquitin Mutant D58A

#### Cat. # UM-D58A

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).

D58 is a residue that has been identified as being important for binding and recognition by proteins that contain Ubiquitin binding domains (UBDs), and represents a new hydrophilic interaction surface on Ubiquitin. This residue may be crucial for the interaction and recognition of poly-Ubiquitin chains by the 26S Proteasome and other enzymes involved in ubiquitination. Ub D58A can form an Ubiquitin-activating (E1) enzyme-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.

#### Product Information

<b>Quantity:</b>	1 mg
<b>MW:</b>	8.6 kDa
<b>Source:</b>	<i>E. coli</i> -derived Accession # P0CG47
<b>Stock:</b>	Lyophilized from a solution of deionized water.
<b>Solubility:</b>	Reconstitute at 10 mg/ml in aqueous buffer.
<b>Purity:</b>	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

## Use & Storage

**Use:** Recombinant Human Ubiquitin Mutant D58A 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). Chains consisting of Recombinant Human Ubiquitin Mutant D58A are unable to interact with and be degraded by the 26S Proteasome. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin Mutant D58A concentration of 0.2-1 mM.

**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. 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. 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.

***For research use only. Not for use in humans.***