
MATERIAL DATA SHEET

Recombinant Human Ubiquitin Mutant L8A

Cat. # UM-L8A

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 has a distinct and functionally important hydrophobic patch whose surface is defined by three residues including Leu8, Ile44 and Val70. These residues are solvent accessible in Ubiquitin chains and are critical for poly-Ubiquitin chain interaction and recognition by the 26S Proteasome and subsequent target degradation. These hydrophobic residues interact directly with various factors that bind to Ubiquitin and/or mediate Ub-Ub interactions in Ubiquitin chains that may influence orientation and recognition. Ubiquitin L8A can form a 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

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

Use & Storage

Use:	Recombinant Human Ubiquitin Mutant L8A 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 L8A 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 L8A 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:

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5. Tong, X. *et al.* (2012) J. Biol. Chem. **287**:25280.
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7. Wertz, I.E. *et al.* (2004) Nature **430**:694.

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