MATERIAL DATA SHEET

Recombinant Human Ubiquitin Mutant F4A
Cat. # UM-F4A

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 two functional surfaces which are critical for various signaling processes. The hydrophobic patch (Leu8, Ile44 and Val70) is important for binding to the proteasome, UIM (Ubiquitin interacting motif) and UBA (Ubiquitin associated) domains. This multifunctional surface thus influences many ubiquitination and deubiquitination reactions. The other surface defined by Phe4 is required specifically for non-proteasome-dependent functions such as endocytosis and internalization, which often involves mono-ubiquitination. It is thought that Phe4 may be involved in specific protein-protein interactions that facilitate endocytosis. In addition, Ile44 forms a di-leucine signal with Leu43 that may be involved in mediating endocytosis of substrate proteins that are mono-ubiquitinated. Ubiquitin F4A 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: *E. coli*-derived
Accession # P0CG47

Stock: Lyophilized from a solution of 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 F4A 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). However, Recombinant Human Ubiquitin Mutant F4A is deficient for Ubiquitin's role in endocytosis and internalization, making it useful for investigating these processes. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin Mutant F4A 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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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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