

MATERIAL DATA SHEET

Recombinant Human Ubiquitin Mutant K27R Cat. # UM-K27R

Ubiquitin is a 76 amino acid (aa) protein that is ubiquitously expressed in all eukaryotic organisms. Ubiquitin is highly conserved with 96% as sequence identity shared between human and yeast Ubiquitin, and 100% as 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 postendocytic sorting (4-7).

Mutation of lysine 27 to arginine renders Ubiquitin (Ub) unable to form poly-Ubiquitin chains via lysine 27 linkages with other Ubiquitin molecules. Ubiquitin K27R 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. Ideal for the reduction in poly-Ubiquitin chain length/conjugation rates and determining if poly-Ubiquitin chains are K27 linked.

Product Information

Quantity: 1 mg

MW: 8.6 kDa

Source: E. coli-derived human Ubiquitin protein

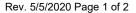
Accession # P0CG47

Stock:

Solubility: Reconstitute at 1-10 mg/mL in an aqueous solution.

Purity: >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal

Coomassie® Blue stain.





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Use & Storage

Use:

The lysine residue utilized for Ubiquitin chain formation is functionally important. Ubiquitin lysine to arginine mutants are ideal for investigating biological processes involving a particular Ubiquitin chain linkage. Recombinant Human Ubiquitin Mutant K27R prevents the formation of K27-linked Ubiquitin chains. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin Mutant K27R concentration of 0.2-1 mM.

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.
- 2. Ciechanover, A. et al. (1980) Proc. Natl. Acad. Sci. USA 77:1365.
- 3. Hershko, 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.
- 8. Spence J., et al. (1995) Mol. Cell. Biol. 15:1265-1273.

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