

MATERIAL DATA SHEET

Recombinant Human His6 Ubiquitin Mutant K33R

Cat. # UM-HK33R

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).

Mutation of lysine 33 to arginine renders Ubiquitin unable to form poly-Ubiquitin chains via lysine 33 linkages with other Ubiquitin molecules. Ubiquitin K33R 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 K33 linked.

Product Information	
Quantity:	1 mg
MW:	9.6 kDa
Source:	<i>E. coli</i> -derived human Ubiquitin protein Contains a 6-His tag Accession # P0CG47
Stock:	Lyophilized from deionized water.
Solubility:	Reconstitute in deionized water or aqueous buffer.
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

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 His6-Ubiquitin Mutant K33R prevents the formation of K33-linked Ubiquitin chains. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human His6-Ubiquitin Mutant K33R concentration of 10-50 μ M.
- 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. 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.

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