

## MATERIAL DATA SHEET

### Recombinant Human Ubiquitin Mutant K11R

#### Cat. # UM-K11R

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 11 to arginine renders Ubiquitin (Ub) unable to form poly-Ubiquitin chains via lysine 11 linkages with other Ubiquitin molecules. Ubiquitin K11R 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 K11-linked.

#### Product Information

<b>Quantity:</b>	1 mg
<b>MW:</b>	8.6 kDa
<b>Source:</b>	<i>E. coli</i> -derived Accession # P0CG47 Contains a Lys-to-Arg substitution at position 11.
<b>Stock:</b>	Lyophilized from a solution in deionized water.
<b>Solubility:</b>	Reconstitute at 10 mg/mL in an aqueous solution.
<b>Purity:</b>	>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 Ubiquitin Mutant K11R prevents the formation of K11-linked Ubiquitin chains. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin Mutant K11R 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:

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7. Wertz, I.E. *et al.* (2004) Nature **430**:694.
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