

MATERIAL DATA SHEET**Ubiquitin Mutant K63R, *human recombinant***
Cat. # UM-K63R

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. 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 Enzyme (E1), 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). 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. Highly purified ubiquitin processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and *in vitro* reactions. Mutation of lysine 63 to arginine renders ubiquitin unable to form polyubiquitin chains via lysine 63 linkages with other ubiquitin molecules. Ub K63R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins (mono-ubiquitination). Ideal for the reduction in polyubiquitin chain length/conjugation rates and determining if polyubiquitin chains are K63 linked.

Product Information

Quantity:	1 mg, lyophilized powder
MW:	8.6 kDa
Solubility:	Reconstitute in aqueous buffers at 5-10 mg/ml
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. Lysine-to-arginine ubiquitin mutants are ideal for investigating biological processes involving specific ubiquitin chain linkages. Recombinant Human Ubiquitin K63R cannot generate K63-linked polyubiquitin chains. Reaction conditions will need to be optimized for each specific application. We recommend an initial ubiquitin K63R concentration of 0.2-1 mM.
Storage:	Store at -20°C after reconstitution. Avoid multiple freeze/thaw cycles.

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Literature

References: Spence J., *et al.* (1995) Mol. Cell. Biol. **15**:1265-1273

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