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MATERIAL DATA SHEET

Recombinant Human Ubiquitin No Lysines, N-Terminal Biotin
Cat. # UB-NOK

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).

Produced via a proprietary process resulting in a single Biotin modification exclusively on the N-terminus of Ubiquitin. This site-specific modification results in a Ubiquitin that is fully functional at the C-terminus. This Ubiquitin mutant lacks all reactive lysine residues which have been mutated to arginine. These mutations render the protein unable to form poly-Ubiquitin chains and can be used as a negative control or to detect mono-ubiquitination. Detection with Avidin-linked reagents allows for a higher efficiency and detection sensitivity than with Anti-Ubiquitin antibodies. Ideal as an alternative to radio-labeled Ubiquitin.

Product Information

Quantity: 50 µg

MW: 9.1 kDa

Source: *E. coli*-derived human Ubiquitin protein
Lacks all reactive lysine residues (mutated to arginine) and contains a single N-terminal biotin
Accession # P0CG47

Stock: X mg/ml (X µM) in 10 mM HEPES pH 7.5

Solubility:

Purity: >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

Use & Storage

Use: Biotinylated Recombinant Human Ubiquitin No-K 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).Biotinylated Recombinant Human Ubiquitin No-K is unable to form chains, making it ideal for use as a negative control for chain formation or to confirm multi-mono-ubiquitination. Reaction conditions will need to be optimized for each specific application. We recommend using Biotinylated Recombinant Human Ubiquitin No-K at a concentration of 10-50 µM. Mono- or multi-ubiquitination can be visualized/quantitated with avidin-linked detection reagents.

Storage: **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 24 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

Literature

References:

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5. Tong, X. *et al*. (2012) J. Biol. Chem. **287**:25280.
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7. Wertz, I.E. *et al*. (2004) Nature **430**:694.

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