MATERIAL DATA SHEET

Recombinant Human Ubiquitin N-Terminal Rhodamine
Cat. # U-600

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 Rhodamine modification exclusively on the N-terminus of Ubiquitin. This site-specific modification results in a Ubiquitin that is fully functional at the C-terminus, and with the full compliment of reactive lysines to allow for poly-Ubiquitin chain incorporation. This reagent allows for poly-Ubiquitin chain incorporation of Rhodamine-N-terminal Ubiquitin with higher efficiency and detection sensitivity than traditionally modified Ubiquitins.

Product Information	
Quantity:	50 µg
MW:	8.9 kDa
Source:	*E. coli*-derived Contains a single N-terminal rhodamine Accession # P0CG47
Stock:	Lyophilized from a solution in HEPES and NaCl.
Solubility:	Reconstitute at 10 mg/mL in an aqueous solution.
Purity:	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.

Use & Storage

Use: Recombinant Human Ubiquitin N-Terminal Rhodamine is ideal for use in assays requiring fluorescent detection. Optimal fluorescence at pH 8.0 is monitored with an excitation wavelength of 570 nm and an emission wavelength of 590 nm. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin N-Terminal Rhodamine concentration of 0.1-1 µM.

Storage: **Protect from light. 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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4. Greene, W. *et al*. (2012) PLoS Pathog. **8**:e1002703.
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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