

MATERIAL DATA SHEET

Recombinant Human Ubiquitin AFC C-Terminal Derivative

Cat. # U-551

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).

Fluorogenic substrate based on the C-terminus derivatization of Ubiquitin with 7-amino-4-trifluoromethylcoumarin (AFC). Similar to Ubiquitin-AMC, this is an exquisitely sensitive deubiquitinating enzyme substrate and is useful for studying Ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring is essential. The fluorophore has a larger Stokes radius than AMC which is useful to reduce compound interference in HTS assays.

Product Information

Quantity:	50 µg
MW:	8.7 kDa
Source:	<i>E. coli</i> -derived Contains a C-terminal AFC Accession # P62988
Stock:	X mg/ml (X µM) in 100% DMSO.
Purity:	>95%, by HPLC.

Use & Storage

- Use:** Recombinant Human Ubiquitin-AFC is a fluorogenic substrate for some Ubiquitin-specific isopeptidases. Release of AFC fluorescence can be monitored with an excitation wavelength of 400 nM and an emission wavelength of 505 nM. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin-AFC concentration of 0.1-1 μ M.
- Storage:** **Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**
- 12 months from date of receipt, -70 °C as supplied.
 - 3 months, -70 °C under sterile conditions after opening.

Literature

References:

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5. Tong, X. *et al.* (2012) J. Biol. Chem. **287**:25280.
6. Wei, W. *et al.* (2004) Nature **428**:194.
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