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## MATERIAL DATA SHEET

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### Recombinant Human Ubiquitin AMC

#### Cat. # U-550

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).

This fluorogenic substrate for Ubiquitin hydrolases is based on the C-terminus derivatization of Ubiquitin with 7-amino-4-methylcoumarin (AMC). Ubiquitin-AMC is an exquisitely sensitive substrate for UCHL3 ( $K_m = 0.039 \mu\text{M}$ ) and for Isopeptidase-T ( $K_m = 0.17 - 1.4 \mu\text{M}$ ). Ubiquitin-AMC is useful for studying Ubiquitin hydrolases when detection sensitivity or continuous monitoring of activity is essential.

#### Product Information

<b>Quantity:</b>	50 $\mu\text{g}$
<b>MW:</b>	8.7 kDa
<b>Source:</b>	<i>E. coli</i> -derived human Ubiquitin protein Accession # P0CG47
<b>Stock:</b>	2.2 mg/ml (250 $\mu\text{M}$ ) in 100% DMSO
<b>Purity:</b>	>95%, by HPLC.

## Use & Storage

**Use:** Recombinant Human Ubiquitin-AMC is a fluorogenic substrate for some Ubiquitin-specific isopeptidases. Release of AMC fluorescence can be monitored with an excitation wavelength of 345 nm and an emission wavelength of 445 nm. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin-AMC concentration of 0.1-1  $\mu$ 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:

1. Sharp, P.M. & W.-H. Li. (1987) Trends Ecol. Evol. **2**:328.
2. Ciechanover, A. *et al.* (1980 ) Proc. Natl. Acad. Sci. USA **77**:1365.
3. Hershko, A. *et al.* (1980) Proc. Natl. Acad. Sci. USA **77**:1783.
4. Greene, W. *et al.* (2012) PLoS Pathog. **8**:e1002703.
5. Tong, X. *et al.* (2012) J. Biol. Chem. **287**:25280.
6. Wei, W. *et al.* (2004) Nature **428**:194.
7. Wertz, I.E. *et al.* (2004) Nature **430**:694.
8. Dang L.C., *et al.* (1998) Biochem. **37**:1868-1879.
9. Mason D.E., *et al.* (2004) Biochem **43**:6535-6544.
10. Stein R.L., *et al.* (1998) Biochem. **34**:12616-12623.

***For research use only. Not for use in humans.***