
MATERIAL DATA SHEET

Recombinant Human Ubiquitin AML C-Terminal Derivative

Cat. # U-556

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).

Substrate for deubiquitinating enzymes based on the C-terminal derivative of Ubiquitin with Aminoluciferin (AML). Rather than fluorescence as the indicator of DUB activity, DUB liberated Luciferin is processed by Luciferase to give a luminescence signal. Ub-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of DUBs that poorly utilize Ub-AMC while using much lower levels of the DUBs themselves.

Product Information	
Quantity:	50 µg
MW:	8.8 kDa
Source:	<i>E. coli</i> -derived Contains a C-terminal Aminoluciferin (AML) Accession # P0CG47
Stock:	Supplied as a solution in HEPES.
Purity:	>95%, by HPLC.

Use & Storage

Use: Recombinant Human Ubiquitin-Aminoluciferin (AML) is ideal for use as a Ubiquitin-specific isopeptidase enzyme substrate. Isopeptidase activity liberates luciferin from Recombinant Human Ubiquitin-Aminoluciferin (AML). ATP and luciferase are then added to produce a luminescent signal proportional to Ubiquitin-specific isopeptidase activity. Optimal luminescence at pH 7.5 can be monitored using all wavelengths with a 500 ms integration time. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin-Aminoluciferin (AML) 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:

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. Herskho, 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.

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