

MATERIAL DATA SHEET

Recombinant Human Ubiquitin Vinyl Methyl Ester C-Terminal Derivative

Cat. # U-203

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).

Ubiquitin-Vinyl Methyl Ester is a potent, irreversible and specific inhibitor of Ubiquitin C-terminal hydrolases (UCHs), Ubiquitin-specific proteases (USPs) and deubiquitinating enzymes (DUBs). It is useful for inhibiting the hydrolysis of poly-Ubiquitin chains on substrate proteins *in vitro* and thus enhances poly-Ubiquitin chain accumulation. Some DUBs, such as those of the JAMM class, are not inhibited by Ubiquitin-Vinyl Methyl Ester.

Product Information

Quantity:	50 µg
MW:	8.6 kDa
Source:	<i>E. coli</i> -derived Contains underivatized and C-terminal Vinyl Methyl Ester derivatized protein, quantity is by derivatized content Accession # P0CG47
Stock:	2.15 mg/ml (250 µM) in 50 mM MES pH 6.0
Purity:	>95%, by HPLC.

Use & Storage

Use: Add Recombinant Human Ubiquitin-Vinyl Methyl Ester (Ub-VME) to *in vitro* assays to inhibit deubiquitinating enzymes. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human Ubiquitin-Vinyl Methyl Ester (Ub-VME) concentration of 1-5 µM.

Storage: 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.

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