

Ubiquitin vinyl methyl ester, (HA-tag)

The ubiquitin cascade in its many forms includes a large family of cysteine proteases known as deubiquitinating enzymes (DUBs). Many members are assigned to this enzyme class by sequence similarity but with little evidence for specific biological activity.

HA-tagged ubiquitin molecules derivatised by chemical ligation at the C-terminus with thiol-reactive functional groups can be used as potent, irreversible inhibitors of DUBs through their covalent modification of the active site and, consequently, can act as specific probes for enzymes with DUB activity. Such probes have been utilized for the identification of DUBs and associated components from cell lysates by proteomic methods, as well as rapid demonstration of enzymatic activity for gene products whose functions were inferred from primary structure.

The nature of the electrophilic group used as the C-terminal derivative, for example vinyl sulfone (-VS), vinyl methylester (-VME), 2-bromoethyl (-Br) or 2-chloroethyl (-Cl), may determine the subset of DUB enzymes detected. The HA peptide sequence (YPYDVPDYA), derived from the influenza hemagglutinin protein, facilitates sensitive identification or purification of HA-Ub-VME modified DUBs through recognition by HA-reactive antibodies in free or agarose-immobilised.

Citations: 2

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Ordering Information

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BML-UW0880-0025	25µg
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Manuals, SDS & CofA

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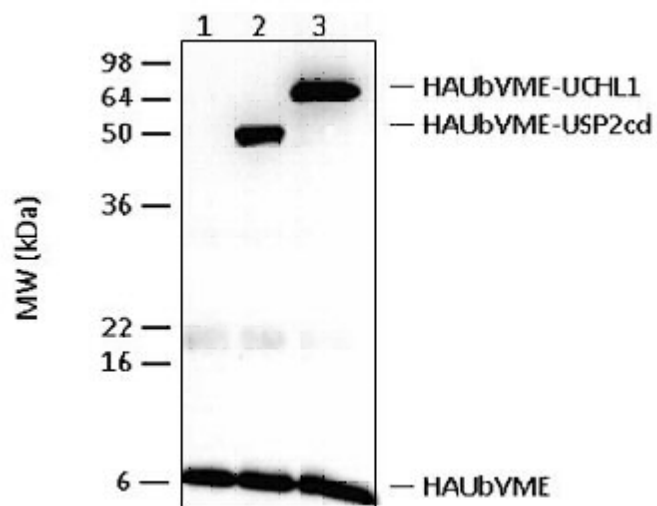


Figure: DUB active site probe assay: Western blot showing reactions containing HA-Ub-VME only (lane 1), HA-Ub-VME + GST-UCHL1 (lane 2, Prod. No. [BML-UW9305](#)), and HA-Ub-VME + USP2cd (lane 3, Prod. No. BML-UW9850). HA-Ub-VME modified proteins detected using HA-reactive polyclonal antibody (Sigma – H6908) at 1:1000 dilution. Typical assay set-up: HA-Ub-VME probe concentration: 0.5-5.0 μ M. Purified enzyme concentration: 0.1-1.0 μ M; Buffer: 50mM TRIS

Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended. Stable for at least 6 months after receipt when stored at -80°C.
Handling	After reconstitution, prepare aliquots, store at -80°C and use within 1 month. Avoid freeze/thaw cycles. After opening, prepare aliquots and store at -80°C.
Short Term Storage	-80°C
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	HA-Ub-VME
Application Notes	Suggested uses: 1. Active site-directed probes for detection of DUBs. 2. Isolation and identification of DUBs. 3. Identification of DUBs within biological samples.
Formulation	Lyophilized.
MW	~10'257Da
Purity	≥95% (HPLC)
Reconstitution	Reconstitute with aqueous buffer up to 5mg/ml (~500µM).
Source	Synthetic.
UniProt ID	P0CG47 (UBB), P0CG48 (UBC), P62979 (RPS27A), P62987 (UBA52)

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