

DUB activity based probe explorer panel (*human, synthetic*)

UbiQ code : UbiQ-L02

Storage : powder at -20°C ; solution at -80°C . Avoid multiple freeze/thaw cycles.

Background. UbiQ-L02 is a panel of 10 deubiquitylating enzyme (DUB) activity based probes¹⁻⁹ (ABPs, 10 ug each) prepared by total chemical synthesis.⁵ These DUB ABPs are potent, irreversible and specific inhibitors of DUBs which can be used for:

- inhibiting hydrolysis of poly-Ub chains on substrate proteins and thus enhancement of poly-Ub chain accumulation.
- structural biology studies of DUB-Ub complexes^{1, 8, 9}
- DUB activity profiling experiments¹⁻⁹
- determine DUB inhibitor specificity^{1, 3, 4}

The panel consists of ABPs with two types of C-terminal warheads: the vinyl methyl ester (VME)³⁻⁹ and the recently developed propargylamide (PA) warhead.^{1, 2} Furthermore various detection/affinity tags are present: the HA and biotin tag^{3, 4, 6-9} and the fluorescent dyes Cy5 and TAMRA^{1, 3} for in-gel fluorescence scanning as read-out.

Ub-VME= UbiQ-005	Ub-PA= UbiQ-057
HA-Ahx-Ahx-Ub-VME= UbiQ-035	HA-Ahx-Ahx-Ub-PA= UbiQ-078
Biotin-Ahx-Ub-VME= UbiQ-054	Biotin-Ahx-Ub-PA= UbiQ-076
TAMRA-Ub-VME= UbiQ-050	TAMRA-Ub-PA= UbiQ-058
Cy5-Ub-VME= UbiQ-071	Cy5-Ub-PA= UbiQ-072

Important: sample preparation.

Dissolve the powder in as little DMSO as possible (*e.g.* 20 mg/mL= 10 ug in 0.5 uL DMSO) and add this DMSO stock slowly to milliQ (please note the order of addition). Next buffer with *e.g.* 1M HEPES to 50 mM HEPES. In general HEPES and Tris buffers are standard for DUB assays - please note that certain DUBs react different to low or high NaCl concentrations. Furthermore, 2-5 mM TCEP or DTT can be used as reducing agent for the DUB (see Wrigley et al. *Cell Biochem. Biophys.* **2011**, 60, 99). A final buffered volume of 20 uL yields a stock of 0.5 mg/mL (± 55 uM) which contains 2.5 vol% DMSO. If required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).

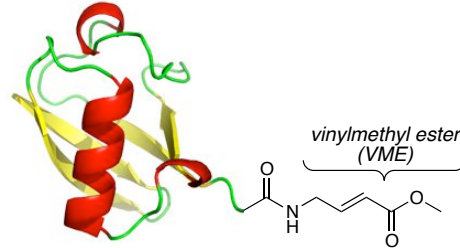
Literature.

1. Ekkebus et al. *J. Am. Chem. Soc.* **2013**, 135, 2867.
2. Sommer et al. *Bioorg. Med. Chem.* **2013**, 21, 2511.
3. de Jong et al. *ChemBioChem* **2012**, 13, 2251.
4. Altun et al. *Chem. Biol.* **2011**, 18, 1401
5. El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, 49, 10149.
6. Misaghi et al. *J. Biol. Chem.* **2005**, 280, 1512.
7. Galardy et al. *Methods in Enzymology* **2005**, 399, 120.
8. Borodovsky et al. *Chemistry and Biology* **2002**, 9, 1149.
9. Borodovsky et al. *EMBO J.* **2001**, 20, 5187.

Note: higher molecular weight artefacts are observed sometimes during SDS-PAGE analysis of monoUb reagents (especially with reactive DUB activity based probes). There is no proof for these higher mol. weight bands actually being present in the material as judged by LC-MS analysis.

Ub-VME (human, synthetic)

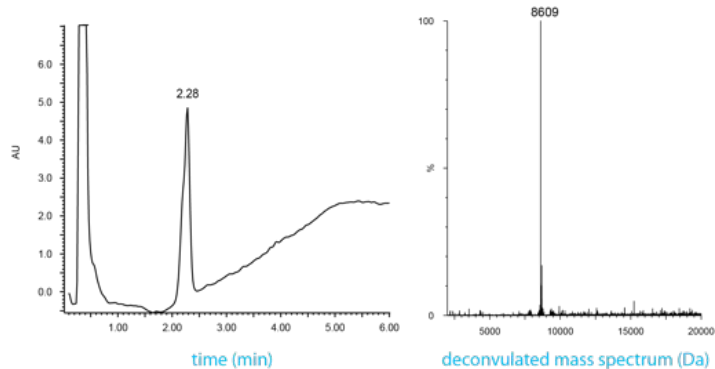
UbiQ code : UbiQ-005
Batch # : B01092012-001
Purity : $\geq 95\%$ by RP-HPLC
Amount : 10 μg , lyophilized powder
Mol. Weight : 8609 Da by MS (calc Mw 8605 Da)



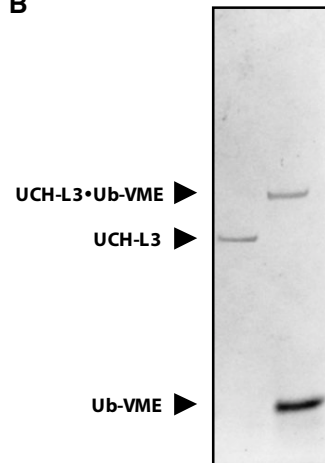
Sequence Ub-VME

MQIFVKLTGKTTITLEVPSDTIENVKAKIQDKGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-VME

A



B



A: LC-MS analysis. Mobile phase A= 1% CH_3CN , 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN . Phenomenex Kinetex C18, (2.1 \times 50 mm, 2.6 μM); flow rate = 0.8 mL/min, column T = 40°C. Gradient: 5% \Rightarrow 95% over 3.5 min. **B: SDS-PAGE analysis** (12% Bis-Tris, MES buffer) of reaction between UCH-L3 and UbiQ-005 (excess). For exp. details, see ref. 1 & 2

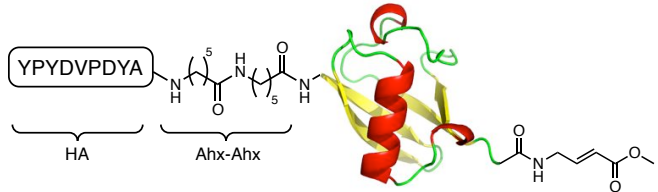
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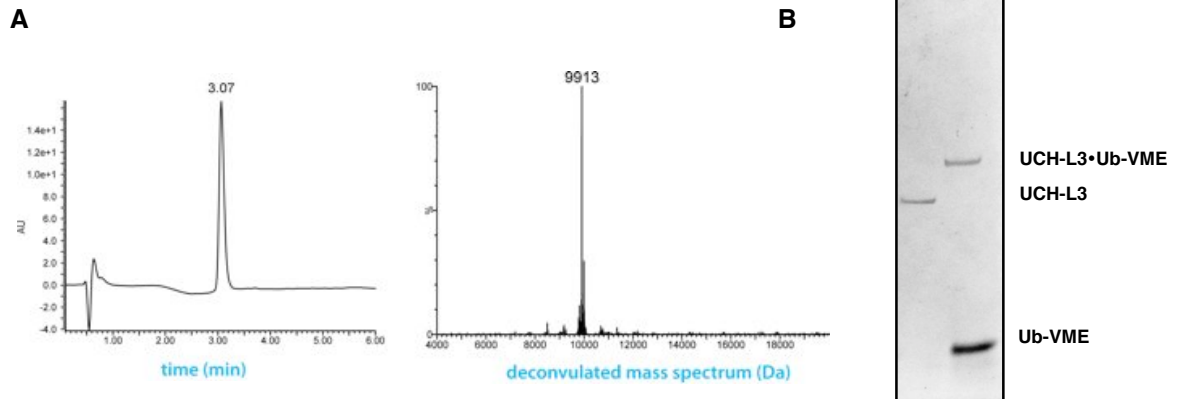
HA-Ahx-Ahx-Ub-VME (human, synthetic)

UbiQ code : UbiQ-035
Batch # : B01042014-001
Amount : 10 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 9913 Da by MS (calc Mw 9912 Da)

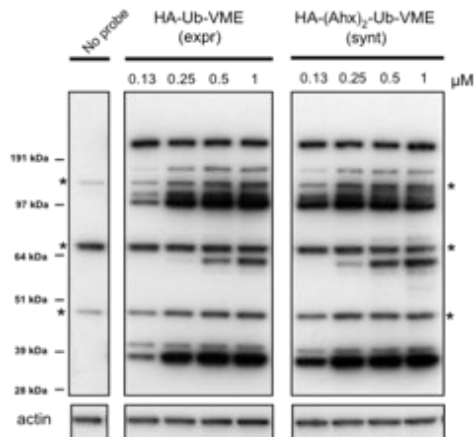


Sequence HA-Ahx-Ahx-Ub-VME

YPYDVPDYA-(Ahx)₂-MQIFVKLTG KTITLVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL EDGRTLSDYN IQKESTLHLV LRLRG-VME



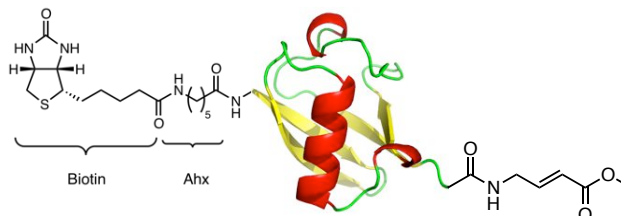
A: LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 μ M; flow rate= 0.8 mL/min, runtime= 6 min, column T= 40°C. Gradient: 0 – 0.5 min: 5% B; 0.5 – 4 min: 5% \Rightarrow 95% B; 4 – 5.5 min: 95% B. **B: SDS-PAGE analysis.** 12% Bis-Tris, MES buffer.



Comparison DUB labeling efficiency between conventional HA-Ub-VME (obtained from bacterial expressed Ub precursor) and synthetic HA-Ahx-Ahx-Ub-VME (UbiQ-035). EL4 cell lysate was incubated at ambient temperature for 15 min. with indicated concentrations of probe; both probes (i.e. expressed and synthetic **UbiQ-035**) showed comparable DUB labeling. *= Background bands due to cross-reactivity of anti-HA antibody.

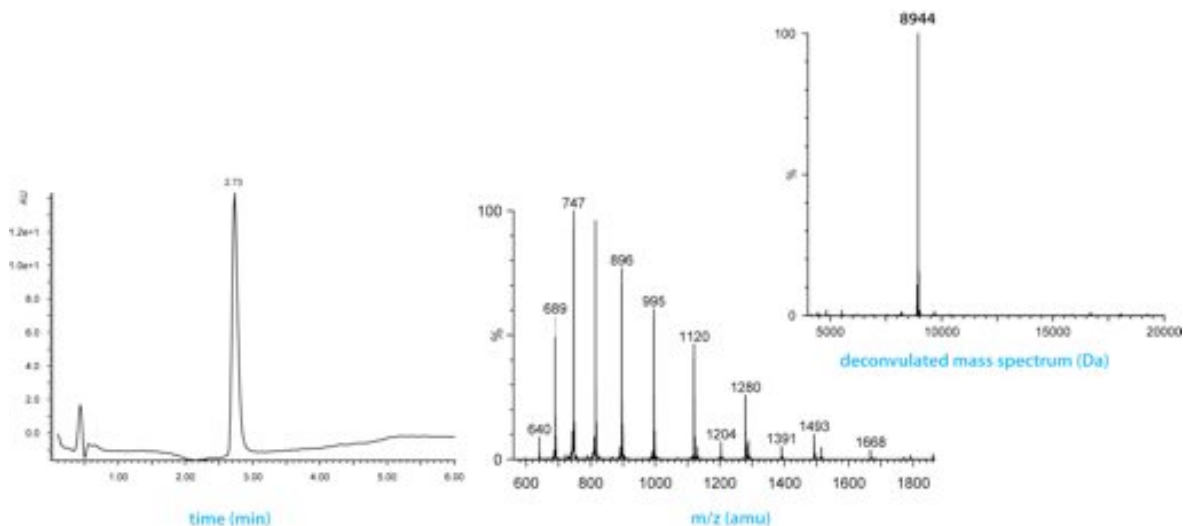
Biotin-Ahx-Ub-VME (*human, synthetic*)

UbiQ code : UbiQ-054
 Batch # : B26112012-001
 Amount : 10 ug, lyophilized powder
 Purity : $\geq 95\%$ by RP-HPLC
 Mol. Weight : found 8944 Da, calc 8945 Da



Sequence Biotin-Ahx-Ub-VME

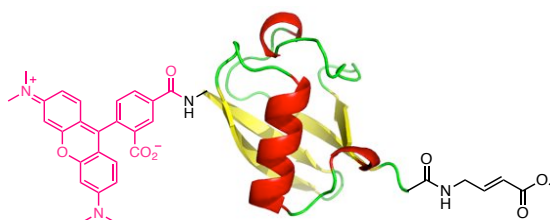
Biotin-Ahx-MQIFVKLTGKTTITLEVPSDTIENVKAKIQDKEGIPPDQQLRIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG-VME



LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.5 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min.

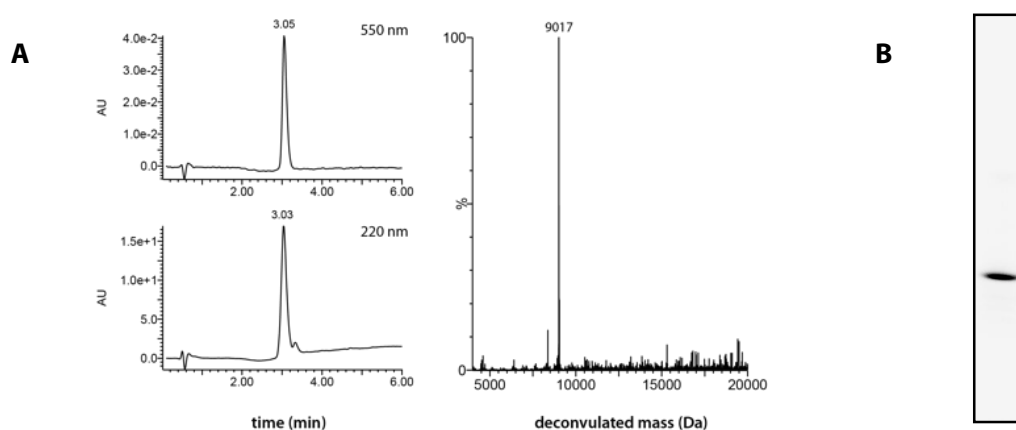
TAMRA-Ub-VME (human, synthetic)

UbiQ code : UbiQ-050
 Batch # : B01072013-001
 Amount : 10 ug, lyophilized powder
 Purity : $\geq 95\%$ by RP-HPLC
 Mol. Weight : 9017 Da by MS (calc Mw 9017 Da)

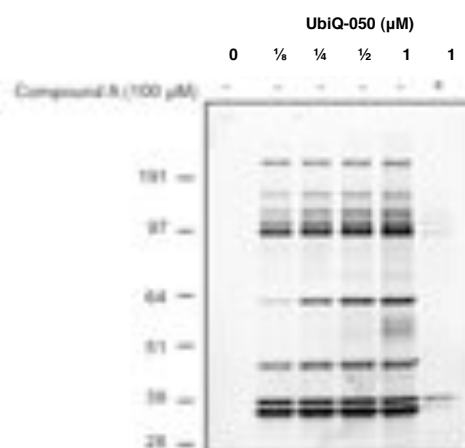


Sequence TAMRA-Ub-VME

TAMRA-MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRG-VME



A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis.** 12% Bis-Tris gel, MES buffer. Fluorescence scan exc 550 nm, emi 590 nm.



Labeling in lysate. EL4 lysate was incubated with indicated concentrations of **UbiQ-050** at ambient temperature for 15 min. Compound **A** is a pan-DUB inhibitor that is included to show how TAMRA-Ub-VME can be used to monitor DUB inhibitor specificity. In-gel fluorescence scans were obtained with a ProXPRESS 2D Proteomic imaging system (Perkin-Elmer) (resolution= 100 μm and exposure time of 60s, $\lambda_{ex}/\lambda_{em}$ = 550/590 nm).

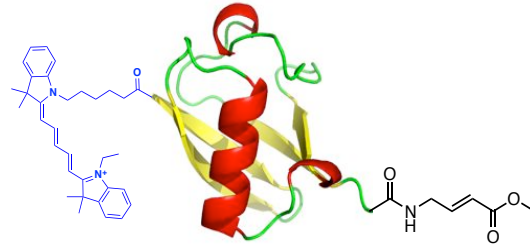
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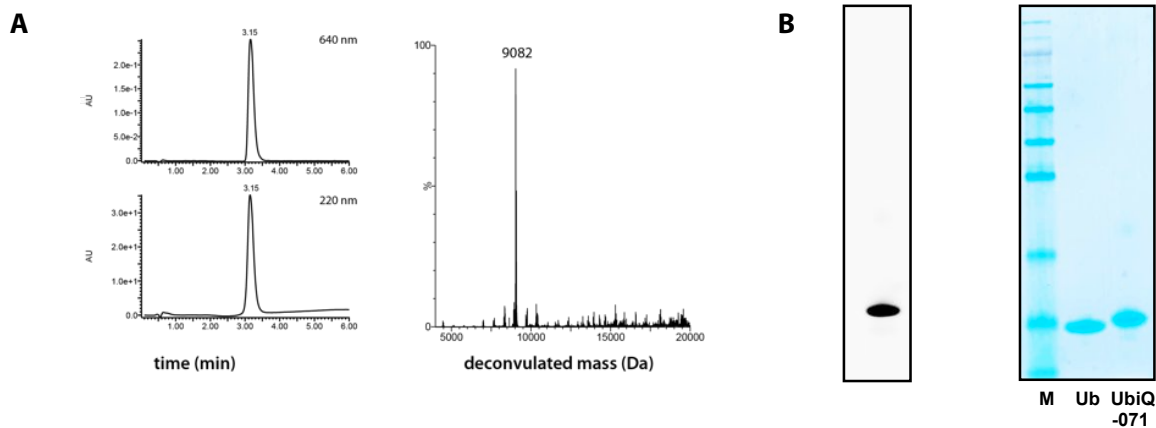
Cy5-Ub-VME (human, synthetic)

UbiQ code : UbiQ-071
Batch # : B01072013-001
Amount : 10 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 9082 Da by MS (calc Mw 9085 Da)



Cy5-Ub-VME

Cy5-MQIFVKLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRG-VME



A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis.** 12% gel, MES buffer. Left: fluorescence scanning (650/690 nm), right: CBB staining.

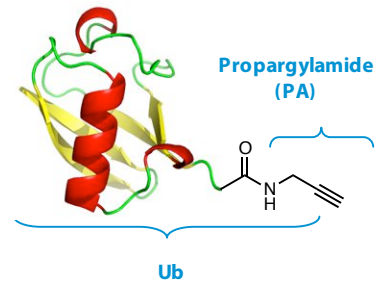
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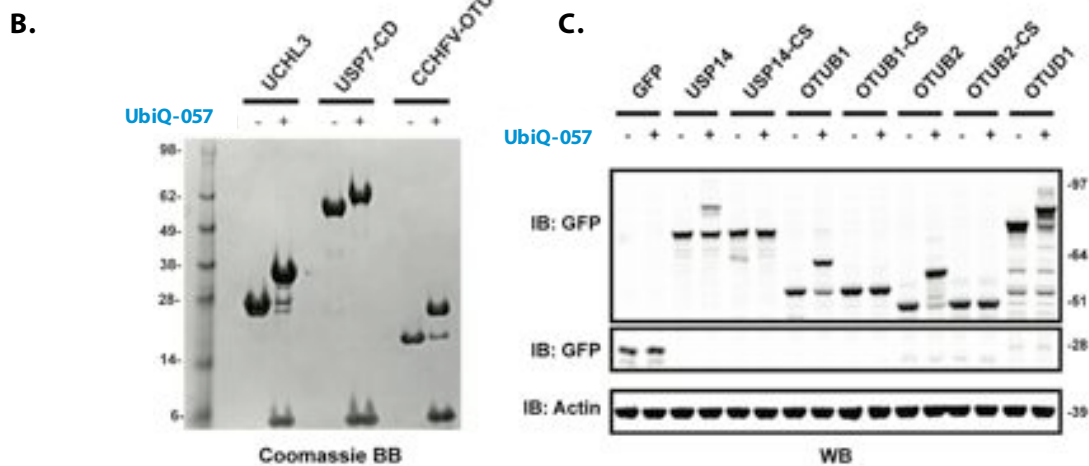
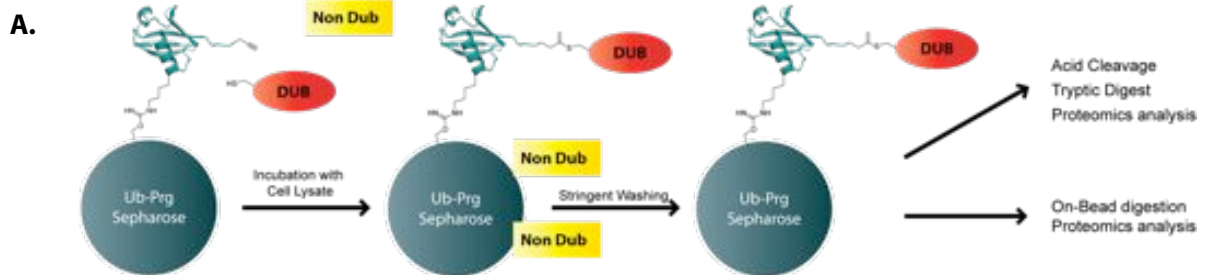
Ub-PA (human, synthetic)

UbiQ code : UbiQ-057
Batch # : B01102012-001
Amount : 10 ug, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 8546 Da by MS (calc Mw 8545 Da)



Sequence Ub-PA

MQIFVKLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-PA



A: Overview of Click-on/Click-off pull down. Ub-Prg can be directly immobilized onto Sepharose resin. The immobilized probe is incubated with a mixture of DUBs and non-DUBs (i.e. lysate). Cysteine DUBs will selectively react with immobilized **UbiQ-057**, resulting in their covalent attachment. Stringent washing removes unbound non-DUBs. After purification, the DUBs can be cleaved under radical conditions for retrieval of active DUBs or by treatment with 5% aq. trifluoroacetic acid for MS-profiling. **B: SDS-PAGE analysis.** *In vitro* reaction of three different classes of DUBs with **UbiQ-057**. **C:** GFP fusions of DUBs from the USP and OTU-classes were transfected in MeJuSo cells and their reaction with **UbiQ-057** visualized using anti-GFP western blot. DUBs annotated with -CS are catalytic Cys to Ser mutants.

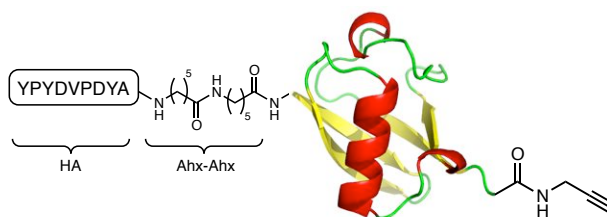
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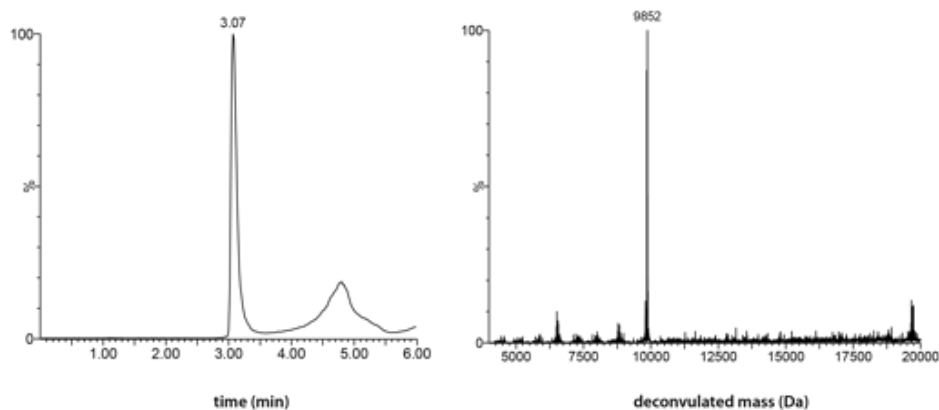
HA-Ahx-Ahx-Ub-PA (*human, synthetic*)

UbiQ code : UbiQ-078
Batch # : B01052014-001
Amount : 10 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 9852 Da by MS (calc Mw 9852 Da)



Sequence HA-Ahx-Ahx-Ub-PA

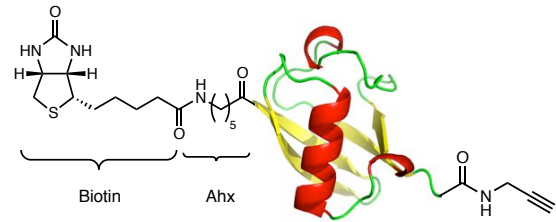
YPYDVDPDYA-Ahx-Ahx-MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-PA



LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 5 μ m 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 30–95% over 3.5 min.

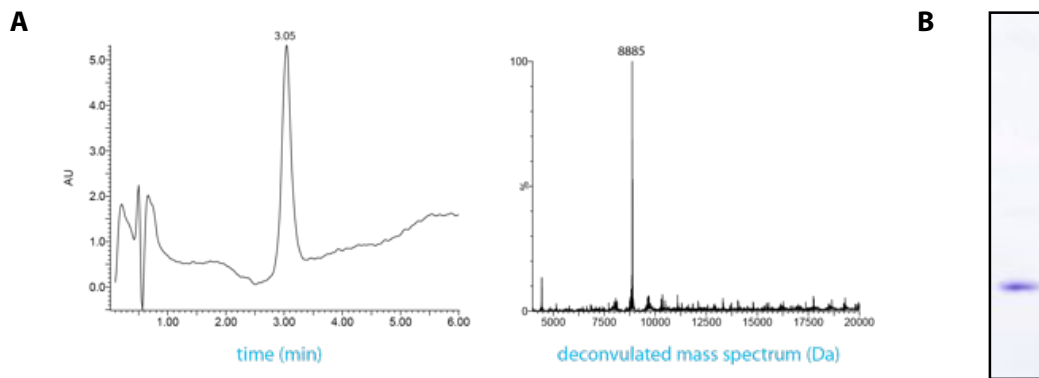
Biotin-Ahx-Ub-PA (human, synthetic)

UbiQ code : UbiQ-076
 Batch # : B01082013-001
 Amount : 10 ug, lyophilized powder
 Purity : $\geq 95\%$ by RP-HPLC
 Mol. Weight : 8885 Da by MS (calc Mw 8885 Da)



Sequence Biotin-Ahx-Ub-PA

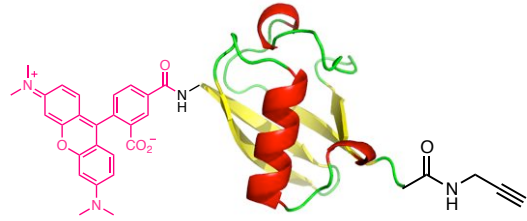
Biotin-Ahx-MQIFVKLTGTITLEVEPSDTIENVKAKIQDKEGIPPDQQRILIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRGG-PA



A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ, B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis.** Coomassie blue staining, 12% SDS-PAGE gel.

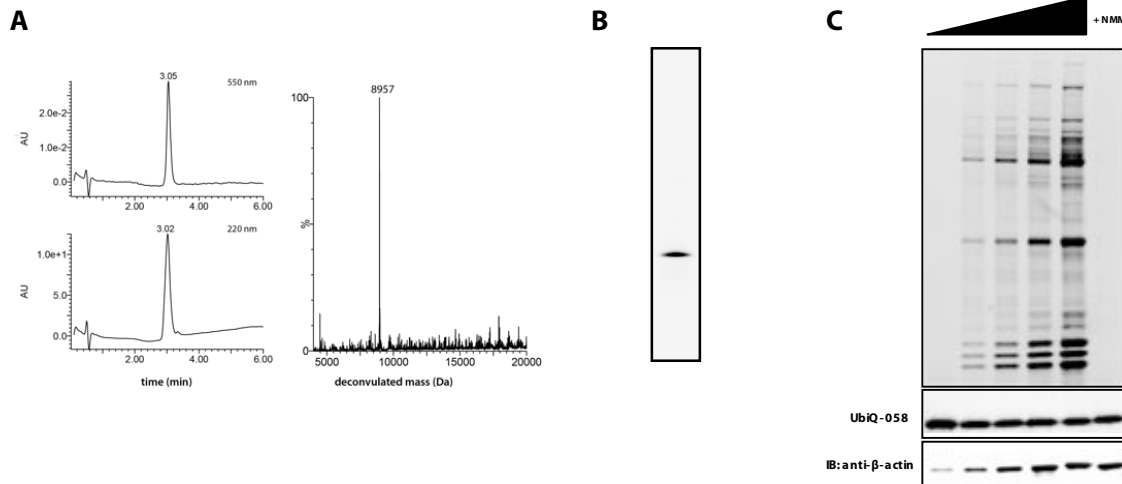
TAMRA-Ub-PA (human, synthetic)

UbiQ code : UbiQ-058
 Batch # : B01072013-001
 Amount : 10 ug, lyophilized powder
 Purity : $\geq 95\%$ by RP-HPLC
 Mol. Weight : 8957 Da by MS (calc Mw 8957 Da)



Sequence TAMRA-Ub-PA

TAMRA-MQIFVKLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-PA



A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1x50 mm, 2.6 μM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis.** 12% gel, MES buffer. Fluorescence scan $\lambda_{ex}/\lambda_{em}$ = 550/590 nm. **C: Cell lysate labeling in lysate.** Labeling of increasing amount of EL4 lysate with **UbiQ-058**.

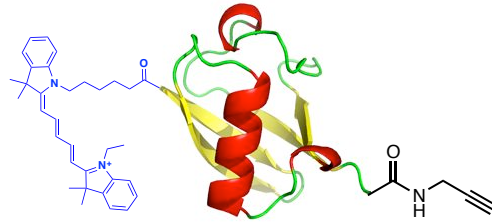
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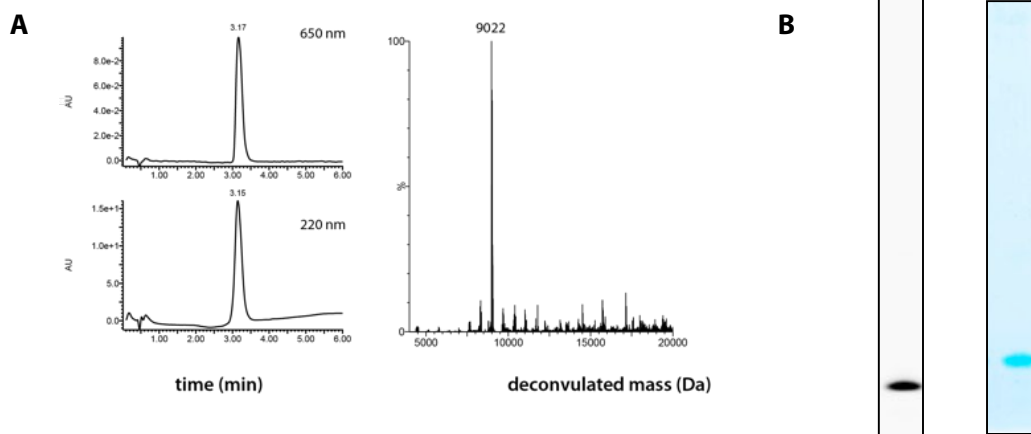
Cy5-Ub-PA (human, synthetic)

UbiQ code : UbiQ-072
Batch # : B01072013-001
Amount : 10 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 8994 Da by MS (calc Mw 9025 Da)



Cy5-Ub-PA

Cy5-MQIFVKLTGKTTITLEVPSDTIENVKAKIQDKGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-PA



A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis,** 12% gel, MES buffer. Left: fluorescence scanning $\lambda_{ex}/\lambda_{em}$ = 650/690 nm, right: CBB staining.