UbiQ
targeting the ubiquitin system

DUB activity-based probe explorer panel

UbiQ code : UbiQ-L02
Storage : upon arrival, powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles.

Background. UbiQ-L02 is a panel of 10 deubiquitylating enzyme (DUB) activity based probes[1-10] (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, 8,10]
- DUB activity profiling experiments[1-10]
- 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
HA-Ahx-Ahx-Ub-VME= UbiQ-035
Biotin-Ahx-Ub-VME= UbiQ-054
TAMRA-Ub-VME= UbiQ-050
Cy5-Ub-VME= UbiQ-071

Ub-PA= UbiQ-057
HA-Ahx-Ahx-Ub-PA= UbiQ-078
Biotin-Ahx-Ub-PA= UbiQ-076
TAMRA-Ub-PA= UbiQ-058
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 as desired (with *e.g.* 1M HEPES to 50 mM HEPES)
- please note that certain DUBs react different to low or high NaCl concentrations.
- TCEP or DTT can be used as reducing agent (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.

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. *Chem Biol* **2002**, *9*, 1149. (9) Borodovsky et al. *EMBO J* **2001**, *20*, 5187. (10) Leestemaker et al. *Methods in Molecular Biology*, *1491*, 113.

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. This can be caused by (heat-induced) aggregation (Morimoto et al. Sci Rep **2018**, 8, article 2711). If possible, avoid heating the samples in Laemmli sample buffer for SDS-PAGE analysis.*

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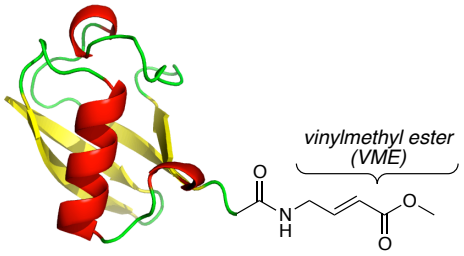


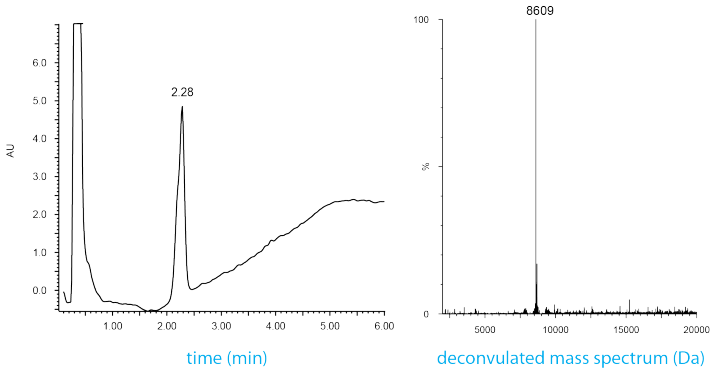
Ub-VME *(human sequence, synthetic)*

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

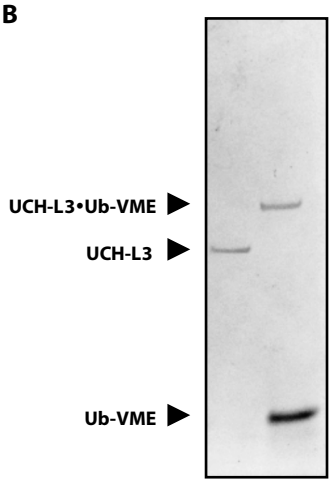
sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**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, (2.1×50 mm, 2.6 µM); flow rate = 0.8 mL/min, column T = 40°C. Gradient: 5% ⇨ 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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UbiQ
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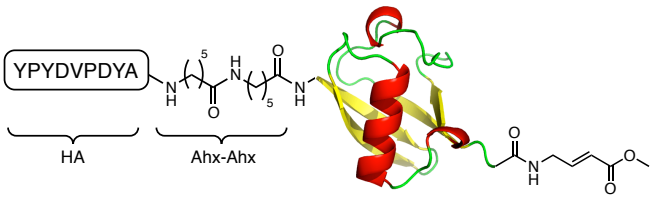


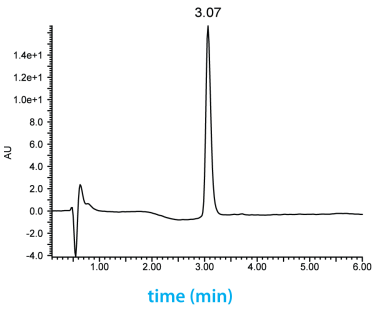
HA-Ahx-Ahx-Ub-VME *(human sequence, synthetic)*

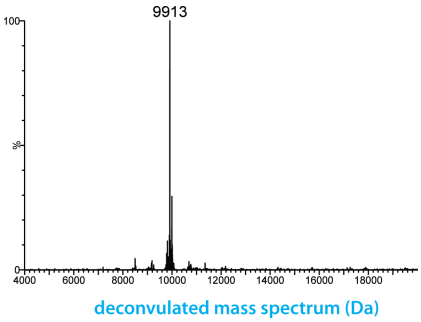
UbiQ code	:	UbiQ-035
Batch #	:	B01042014-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9913 Da by MS (calc Mw 9912 Da)

sequence

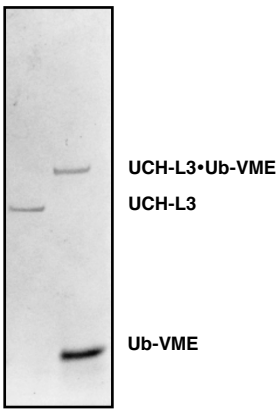
YPYDVPDYA-**(Ahx)$_2$**-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

A

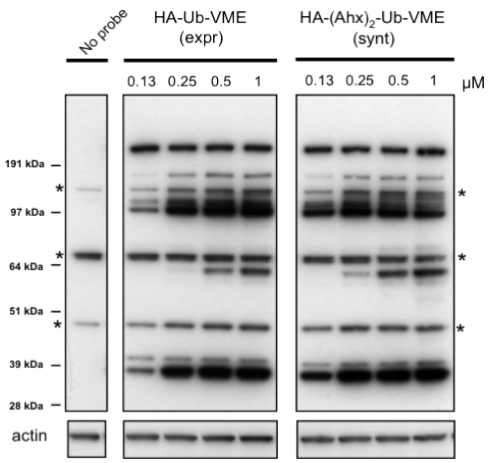




B



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 CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 µM); 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% ⇨ 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.

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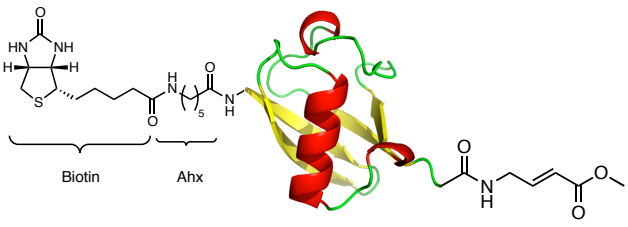
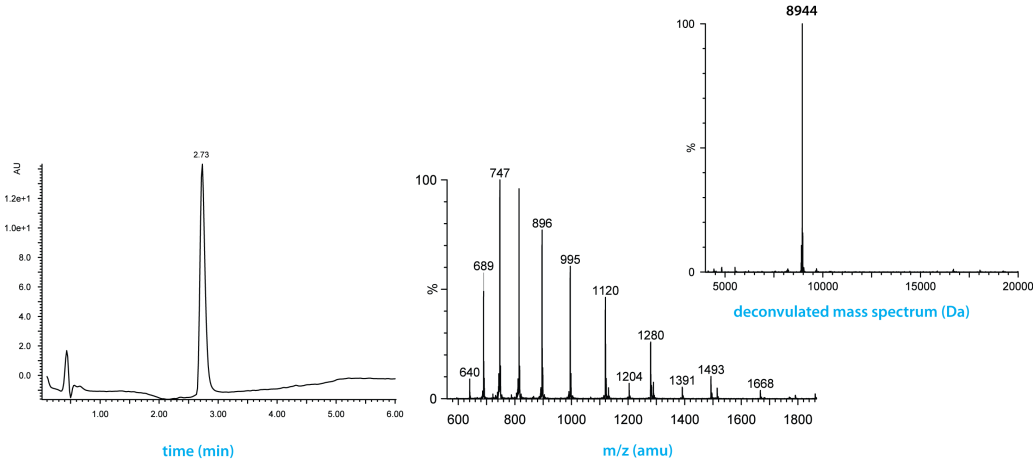


Biotin-Ahx-Ub-VME *(human sequence, synthetic)*

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

sequence

Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**



LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. 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.

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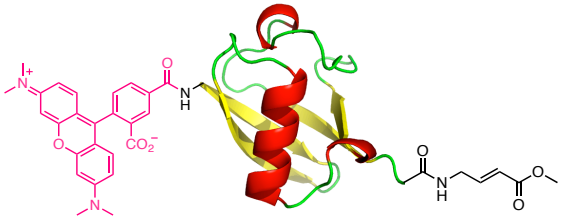


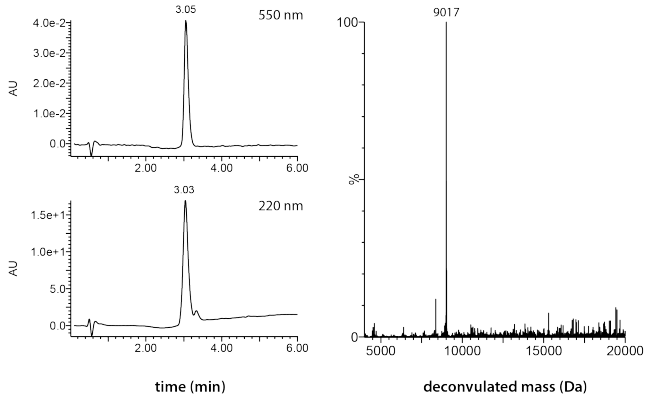
TAMRA-Ub-VME *(human sequence, synthetic)*

UbiQ code	:	UbiQ-050
Batch #	:	B01072013-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.02 kDa

sequence

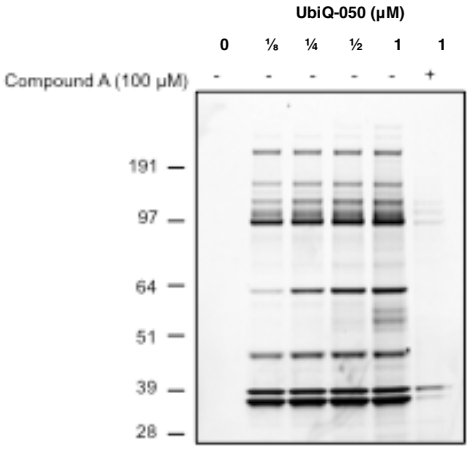
TAMRA-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

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, (2.1×50 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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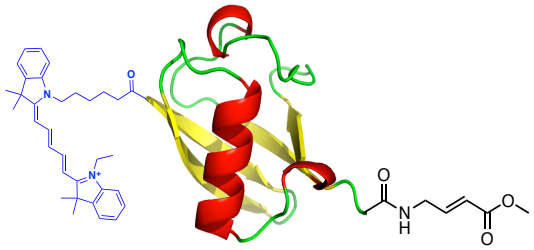
Cy5-Ub-VME *(human sequence, synthetic)*

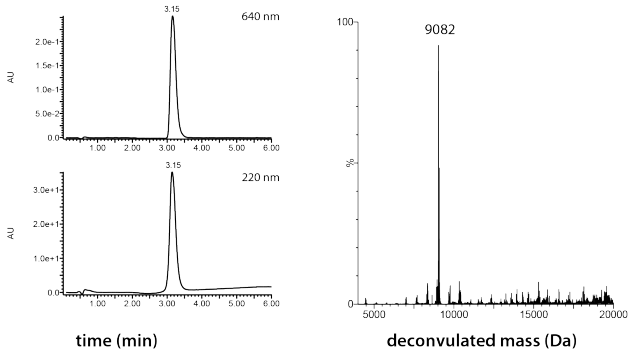
UbiQ code	:	UbiQ-071
Batch #	:	B01072013-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9082 Da by MS (calc Mw 9085 Da)



Cy5-Ub-VME

Cy5-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

A



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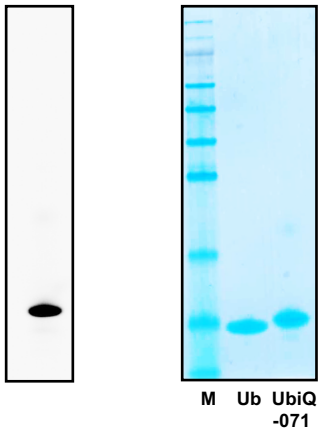

A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇨ 95% over 3.5 min. **B: SDS-PAGE analysis.** 12% gel, MES buffer. Left: fluorescence scanning (650/690 nm), right: CBB staining.

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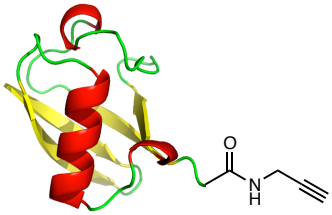


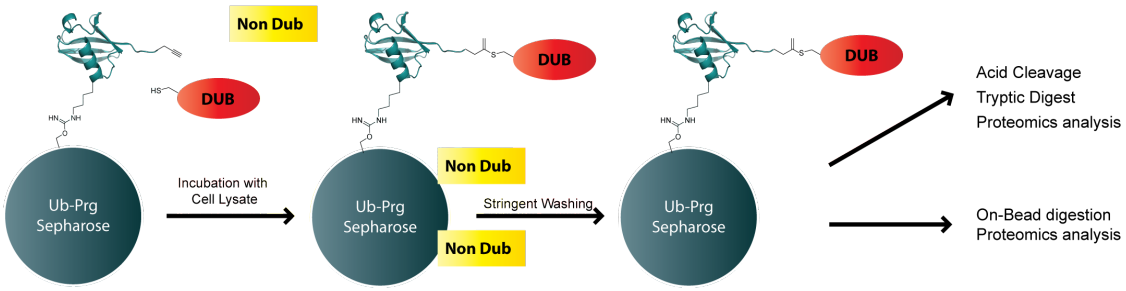
Ub-PA *(human sequence, synthetic)*

UbiQ code	:	UbiQ-057
Batch #	:	B01102012-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	8.55 kDa

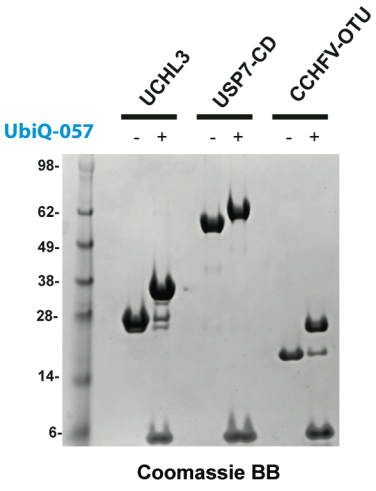
sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

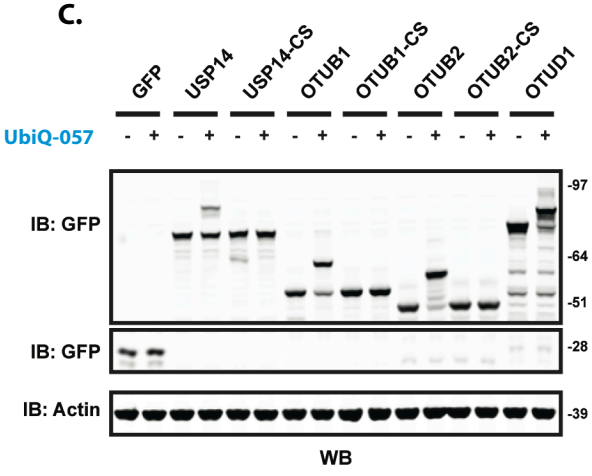
A.



B.



C.



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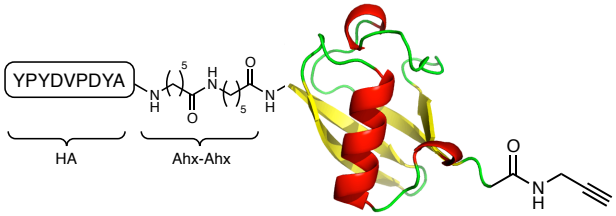
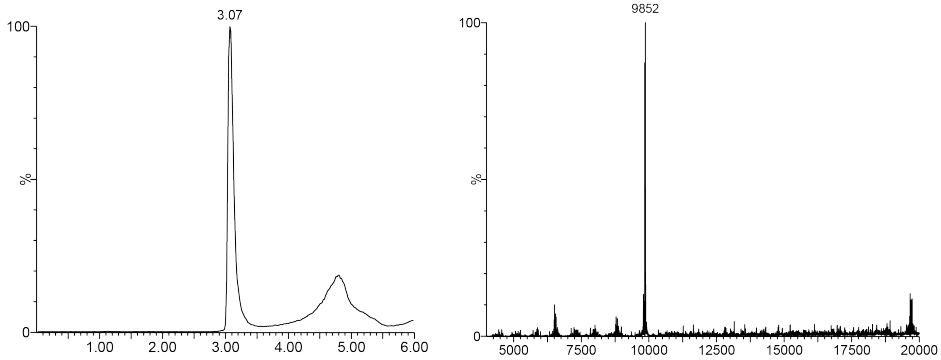
HA-Ahx-Ahx-Ub-PA *(human sequence, synthetic)*

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



sequence

YPYDVPDYA-Ahx-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**



LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 30–95% over 3.5 min.

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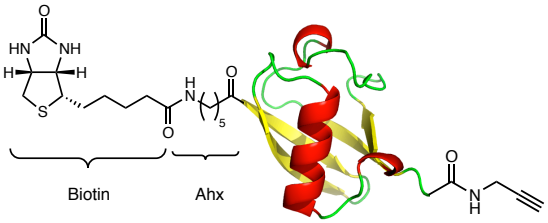


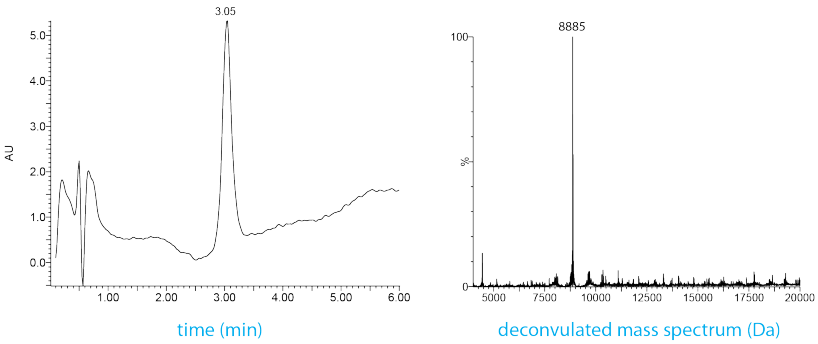
Biotin-Ahx-Ub-PA *(human sequence, synthetic)*

UbiQ code	:	UbiQ-076
Batch #	:	B01082013-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	8.89 kDa

sequence

Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

A



B

A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ, B= 1% milliQ and 0.1% formic acid in CH_3CN. 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.** Coommassie blue staining, 12% SDS-PAGE gel.

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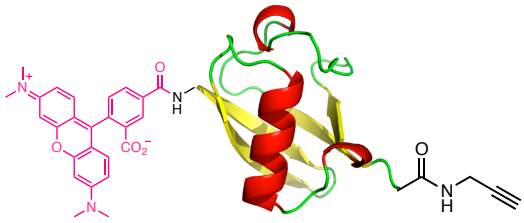
TAMRA-Ub-PA *(human, synthetic)*

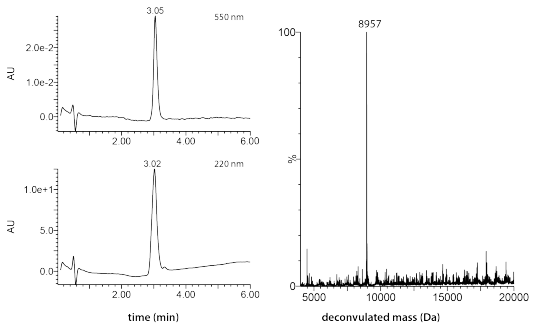
UbiQ code	:	UbiQ-058
Batch #	:	B01072013-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	8.96 kDa



sequence

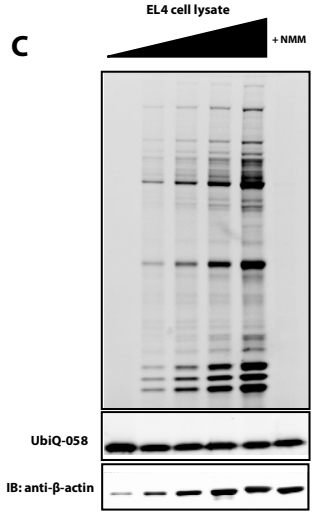
TAMRA-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

A



B

C



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, (2.1×50 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. Fluorescence scan $\lambda_{ex}/\lambda_{em}$= 550/590 nm. **C:** Labeling of increasing amounts of EL4 lysate with **UbiQ-058**.

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BIC/SWIFT RABONL2U

UbiQ
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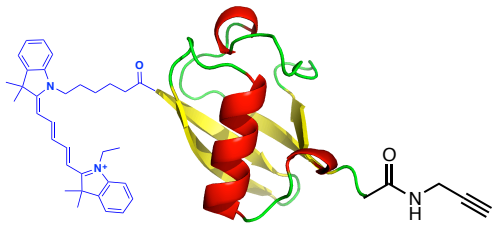


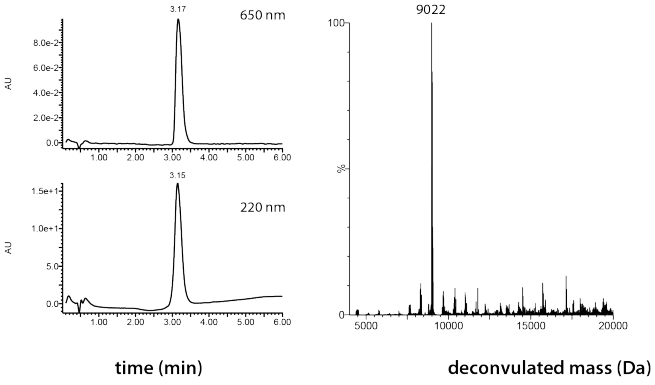
Cy5-Ub-PA *(human sequence, synthetic)*

UbiQ code	:	UbiQ-072
Batch #	:	B01072013-001
Amount	:	10 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.0 kDa

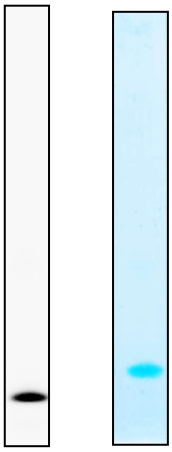
Cy5-Ub-PA

Cy5-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

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, (2.1×50 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.

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