

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 probes¹⁻¹⁰ (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 experiments¹⁻¹⁰
- 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-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-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).

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 and/or add 4M urea to the loading buffer.

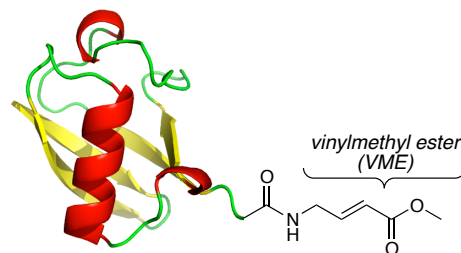
Literature. (1) Ekkebus et al. *J Am Chem Soc* **2013**, 135, 2867. (2) Sommer et al. *Bioorg Med Chem* **2013**, 21, 2511. (3) de Jong et al. *ChemBioChem* **2012**, 13, 2251. (4) Altun et al. *Chem Biol* **2011**, 18, 1401. (5) El Oualid et al. *Angew Chem Int Ed* **2010**, 49, 10149.

UbiQ

targeting the ubiquitin system

Ub-VME (human sequence, synthetic)

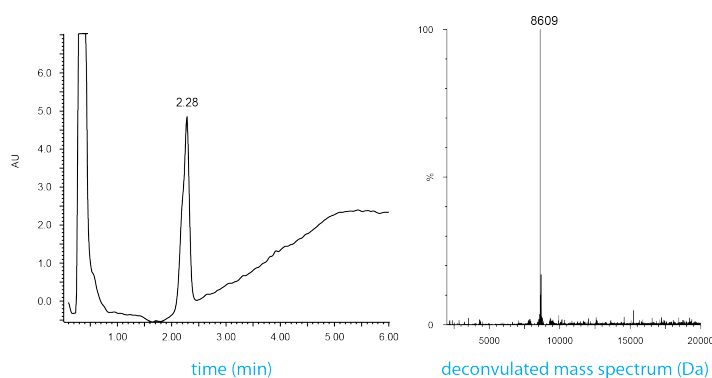
UbiQ code : UbiQ-005
 Batch # : B01092012-001
 Purity : ≥95% by RP-HPLC
 Amount : 10 ug, lyophilized powder
 Mol. Weight : 8.61 kDa



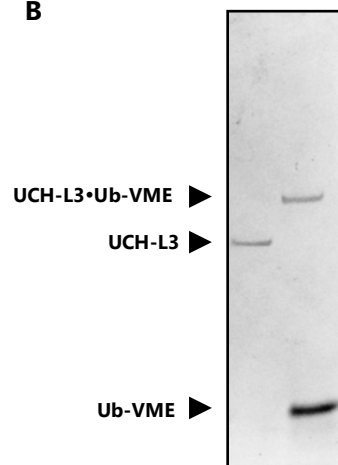
sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQORLIFAGKQLEDGRTLSDYNIQKESTLHLVLR^{LG}-VME

A



B



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= 0.8 mL/min, column T= 40°C. Gradient: 5-95%B 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

UbiQ

targeting the ubiquitin system

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

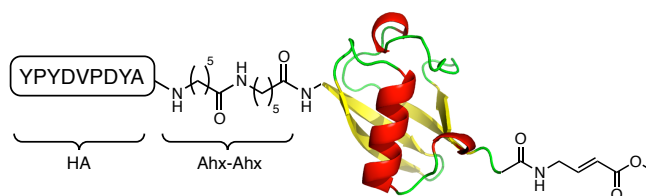
UbiQ code : UbiQ-035

Batch # : B01042014-001

Amount : 10 ug, lyophilized powder

Purity : ≥95% by RP-HPLC

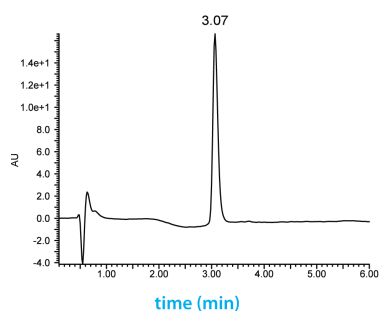
Mol. Weight : 9.91 kDa



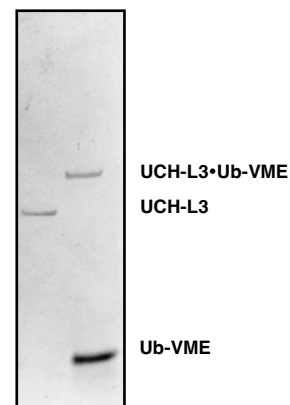
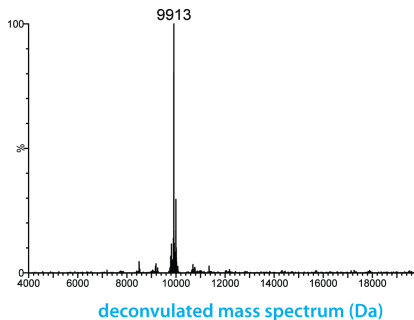
sequence

YPYDVDPYA-(Ahx)₂-MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRG-VME

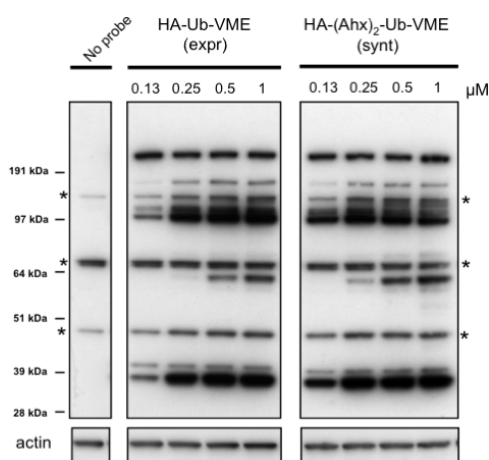
A



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 CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 μm; flow rate= 0.8 mL/min, runtime= 6 min, column T= 40°C. Gradient: 5-95%B over 3.5 min. **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.

UbiQ Bio BV

Chamber of Commerce 50023438
VAT NL822502136B01

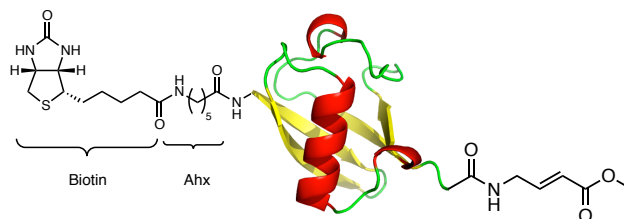
Science Park 301
1098 XH Amsterdam
The Netherlands

t +31 20 303 1970
e info@ubiqbio.com
i www.ubiqbio.com

Rabobank
IBAN: NL86 RABO 0150658907
BIC/SWIFT: RABONL2U

UbiQ

targeting the ubiquitin system



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

UbiQ code : UbiQ-054

Batch # : B26112012-001

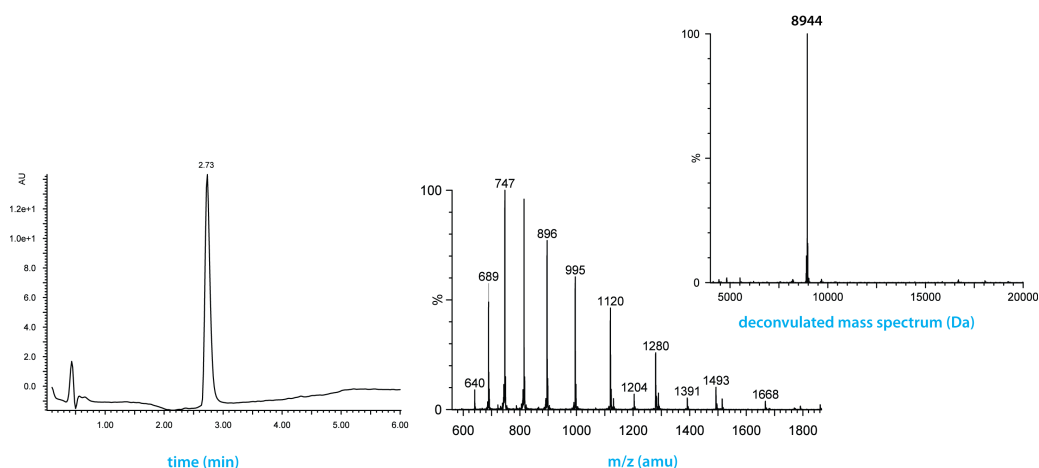
Amount : 10 ug, lyophilized powder

Purity : ≥95% by RP-HPLC

Mol. Weight : 8.94 kDa

sequence

Biotin-Ahx-MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPDPQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLR-LRG-VME



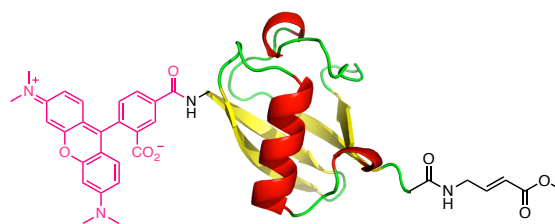
LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate= 0.5 mL/min, runtime = 6 min, column T= 40°C. Gradient: 5-95%B over 3.5 min.

UbiQ

targeting the ubiquitin system

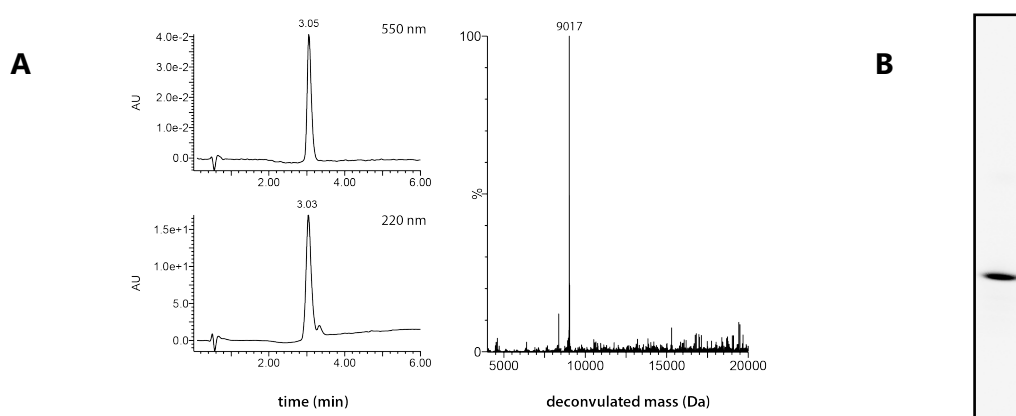
TAMRA-Ub-VME (human sequence, synthetic)

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

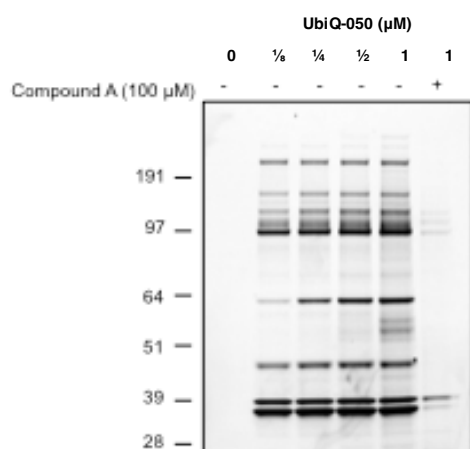


sequence

TAMRA-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLR-LRG-**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%B 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).

UbiQ

targeting the ubiquitin system

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

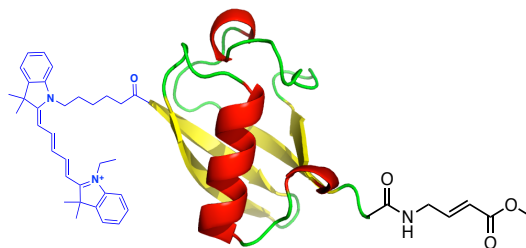
UbiQ code : UbiQ-071

Batch # : B01072013-001

Amount : 10 ug, lyophilized powder

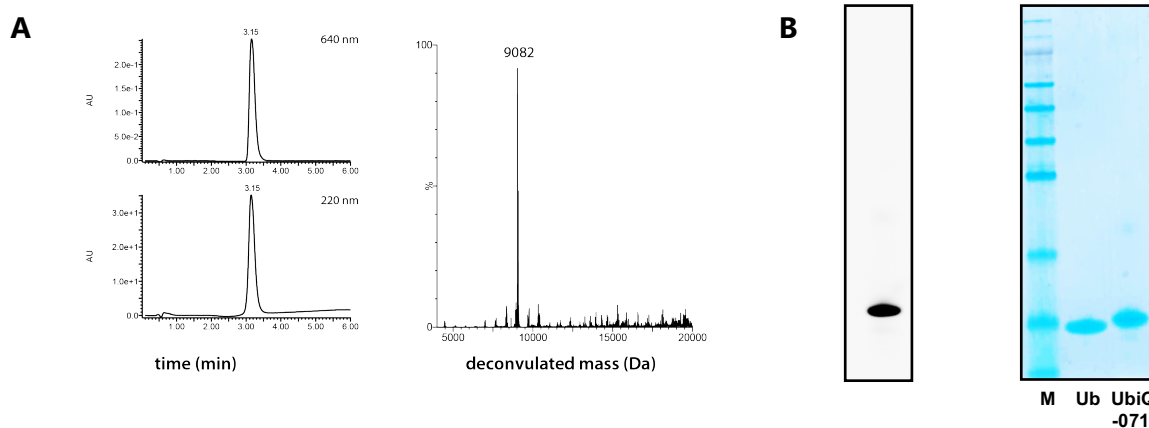
Purity : ≥95% by RP-HPLC

Mol. Weight : 9.08 kDa



Cy5-Ub-VME

Cy5-MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRG-**VME**



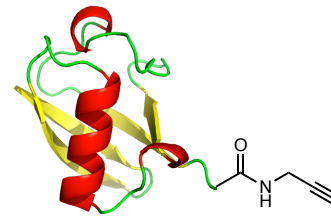
A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5-95%B over 3.5 min. **B: SDS-PAGE analysis.** 12% gel, MES buffer. Left: fluorescence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

UbiQ

targeting the ubiquitin system

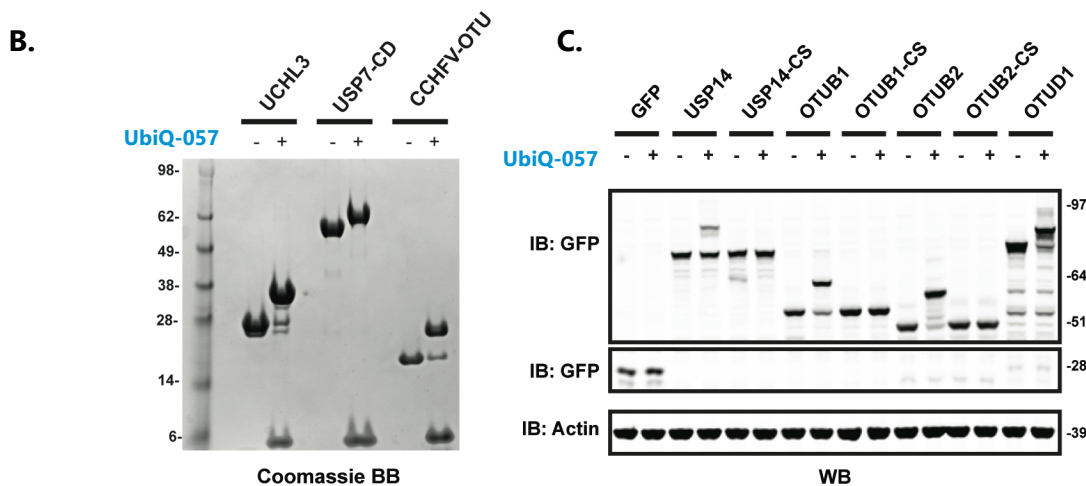
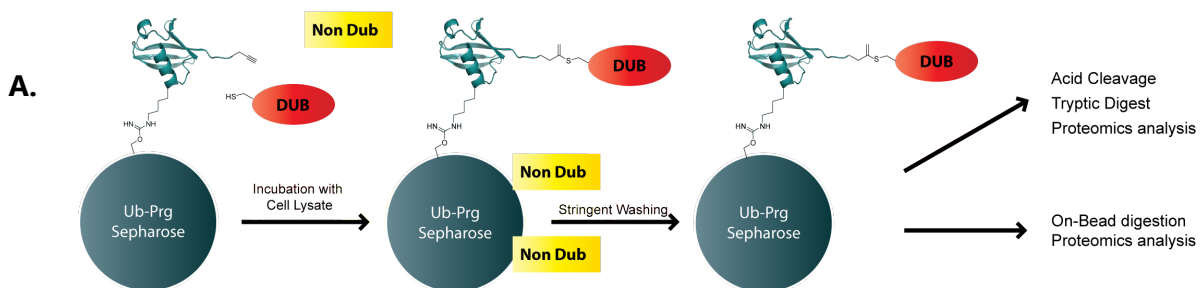
Ub-PA (human sequence, synthetic)

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



sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRG-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.

UbiQ

targeting the ubiquitin system

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

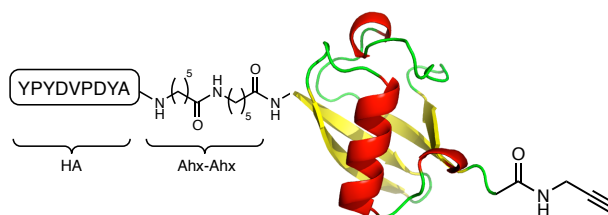
UbiQ code : UbiQ-078

Batch # : B01052014-001

Amount : 10 ug, lyophilized powder

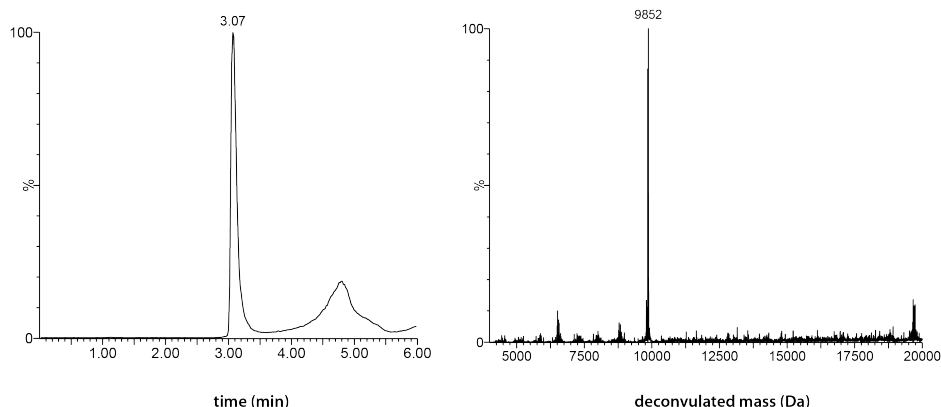
Purity : ≥95% by RP-HPLC

Mol. Weight : 9.85 kDa



sequence

YPYDVDPYA-Ahx-Ahx-MQIFVKLTITGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRG-PA



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

UbiQ

targeting the ubiquitin system

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

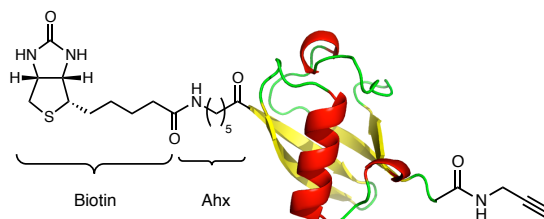
UbiQ code : UbiQ-076

Batch # : B01082013-001

Amount : 10 ug, lyophilized powder

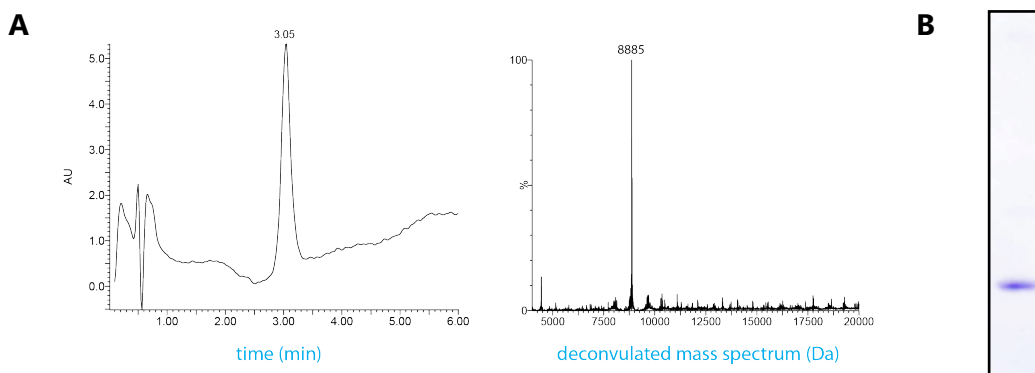
Purity : $\geq 95\%$ by RP-HPLC

Mol. Weight : 8.89 kDa



sequence

Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRG-PA



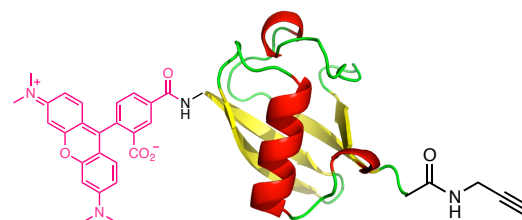
A: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ, B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5-95%B over 3.5 min. **B: SDS-PAGE analysis.** Coomassie blue staining, 12% SDS-PAGE gel.

UbiQ

targeting the ubiquitin system

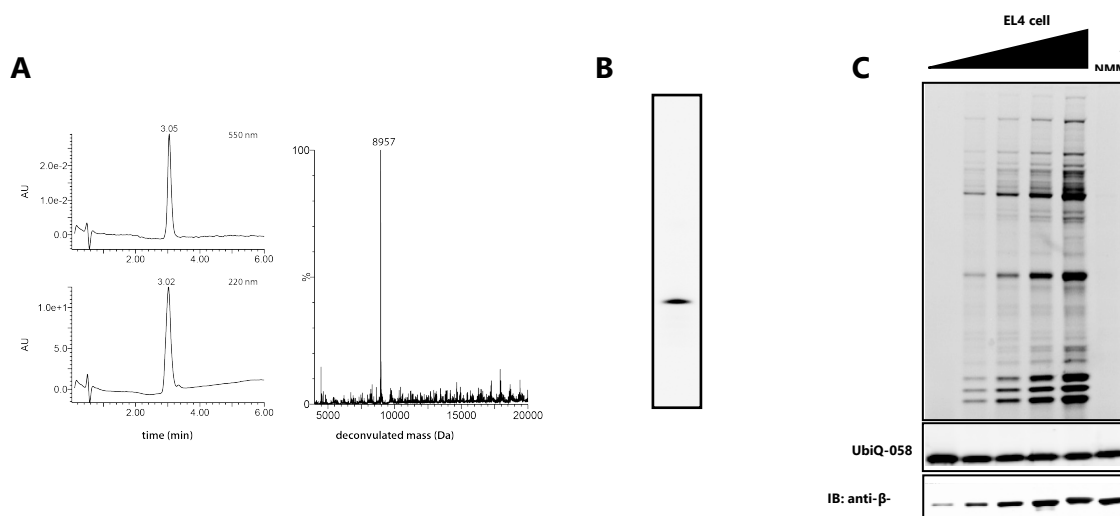
TAMRA-Ub-PA (human, synthetic)

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



sequence

TAMRA-MQIFVKTLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-**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%B 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.

UbiQ

targeting the ubiquitin system

Cy5-Ub-PA (human sequence, synthetic)

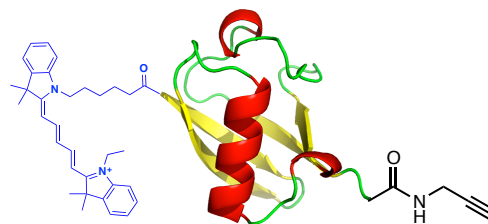
UbiQ code : UbiQ-072

Batch # : B01072013-001

Amount : 10 ug, lyophilized powder

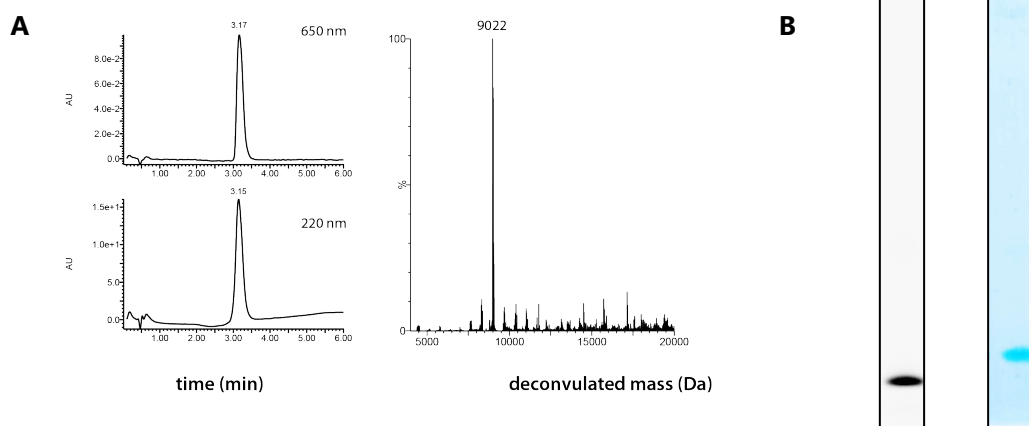
Purity : $\geq 95\%$ by RP-HPLC

Mol. Weight: 9.02 kDa



Cy5-Ub-PA

Cy5-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, (2.1×50 mm, 2.6 μM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5-95%B 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.