UbiQ
targeting the ubiquitin system

DUB assay explorer panel

UbiQ code: UbiQ-L03
Amount : 4 x 25 µg lyophilized powder
Purity : ≥95% (purified by RP-HPLC)
Storage : upon arrival, powder at −20°C, solution at −80°C. Please avoid multiple freeze/thaw cycles and protect from

Productsheet

Background. UbiQ-L03 is a panel of 4 deubiquitinating enzyme (DUB) activity assay reagents (25 ug each), prepared by total chemical synthesis.[1]

- **Ub-AMC (UbiQ-001)** is a quenched fluorogenic DUB substrate based on the C-terminal derivatization of ubiquitin with 7-amido-4-methylcoumarin (AMC). Cleavage of the amide bond between Gly76 of ubiquitin and the AMC moiety results in an increase in fluorescence at λ_{ex} / λ_{ex}= 380 / 460 nm.[2]

- **Ub-Rh110Gly (UbiQ-002)** is a quenched fluorogenic DUB substrate based on the C-terminal derivatization of ubiquitin with rhodamine110-Gly (Rh110Gly). Cleavage of the amide bond between Ub Gly76 and Rh110Gly results in an increase in fluorescence at λ_{ex} / λ_{ex}= 535 / 485 nm.[3]

- **TAMRA-Lys(Ub)-Gly (UbiQ-012)** is a fluorescence polarization assay reagent based on a 5-carboxytetramethylrhodamine (TAMRA, λ_{ex} / λ_{ex}= 530 / 580 nm) modified Lys-Gly sequence that is linked to ubiquitin via a native isopeptide bond with the lysine side-chain.[4]

- **Ub-aminoluciferin (UbiQ-036)** is a DUB substrate based on the C-terminal derivatization of ubiquitin with 6-aminoluciferin (Luc). Upon cleavage by a DUB, the released 6-aminoluciferin functions as a substrate for luciferase, allowing detection of luminescence as a read-out for DUB-activity.[5]

important - sample preparation.

- dissolve the powder in as little DMSO as possible (*e.g.* 25 mg/mL= 25 ug in 1 uL DMSO)
- add this DMSO stock slowly to milliQ (please note the order of addition)
- next buffer as desired (*e.g.* with 1M HEPES to 50 mM HEPES)
- for example, a stock of 100 uM in buffer (±0.9 mg/mL, ±4vol% DMSO) is diluted 1000× to prepare a final standard assay solution of 100 nM substrate concentration

Literature. (1) El Oualid et al. *Angew Chem Int Ed* **2010**, 49, 10149. (2a) Dang et al. *Biochemistry* **1998**, 37, 1868. (2b) Mason et al. *Biochemistry* **2004**, *43*, 6535. (3) Hassiepin et al. *Analyt Biochem* **2007**, *371*, 201. (4a) Huang et al. *Methods in Molecular Biology* **2009**, *565*, 127. (4b) Geurink et al. *ChemBiochem*, **2012**, *13*, 293. (5a) White et al. *J Am Chem Soc* **1966**, *88*, 2015. (5b) Reddy et al. *J Am Chem Soc* **2010**, *132*, 13586. (5c) Orcutt et al. *Biochim Biophys Acta* **2012**, *1823*, 2079.

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

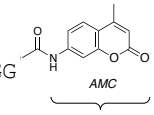
UbiQ
targeting the ubiquitin system

Ub-AMC *(human sequence, synthetic)*

UbiQ code	:	UbiQ-001
Batch #	:	B01012013-001
Purity	:	≥95% by RP-HPLC
Amount	:	25 ug, lyophilized powder
Mol. Weight	:	8.72 kDa

A

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG



B

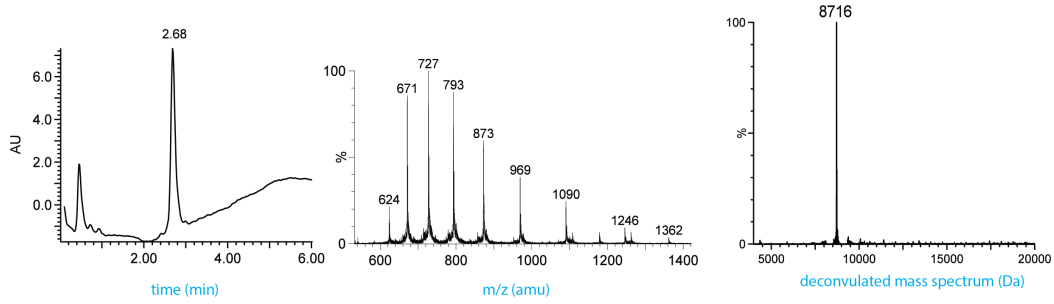


Figure 1. UbiQ-001: A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.8 mL/min, column T= 40°C. Gradient: 5-95% over 3.5 min.

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

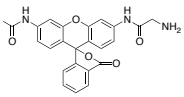
Rabobank
IBAN: NL86 RABO 0150658907
BIC/SWIFT: RABONL2U

UbiQ
targeting the ubiquitin system

Ub-Rh110Gly *(human sequence, synthetic)*

UbiQ code	:	UbiQ-002
Batch #	:	B01092013-001
Purity	:	≥95% by RP-HPLC
Amount	:	25 ug, lyophilized powder
Mol. Weight	:	8.93 kDa

A

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG 

B

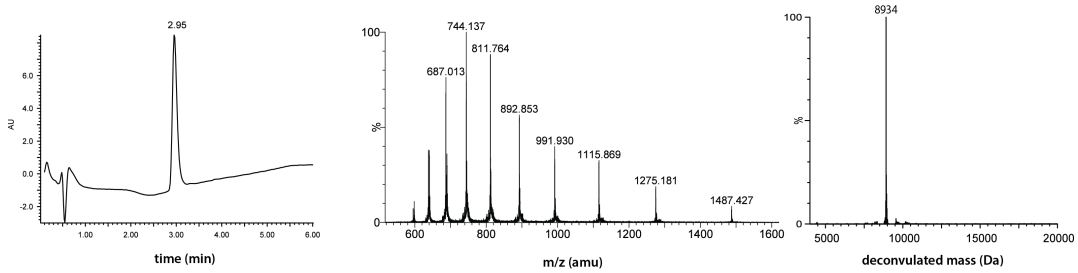


Figure 2. UbiQ-002: A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.8 mL/min, column T= 40°C. Gradient: 5-95% over 3.5 min.

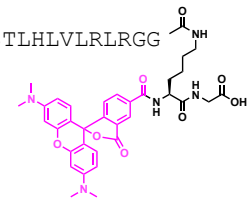
UbiQ
targeting the ubiquitin system

5-TAMRA-Lys(Ub)-Gly-OH *(human sequence, synthetic)*

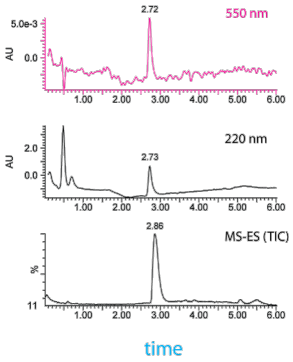
UbiQ code : UbiQ-012
Batch # : B24012013-001
Purity : ≥95% by RP-HPLC
Amount : 25 ug, lyophilized powder
Mol. Weight: 9.16 kDa

A

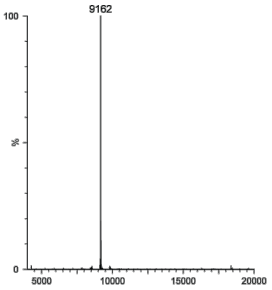
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG



B



time



deconvulated mass spectrum

Figure 3. UbiQ-012: A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water 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.

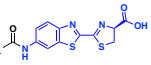
UbiQ
targeting the ubiquitin system

Ubiquitin-aminoluciferin *(human sequence, synthetic)*

UbiQ code	:	UbiQ-036
Batch #	:	B01092013-001
Purity	:	≥95% by RP-HPLC
Amount	:	25 ug, lyophilized powder
Mol. Weight	:	8.82 kDa

A

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG



B

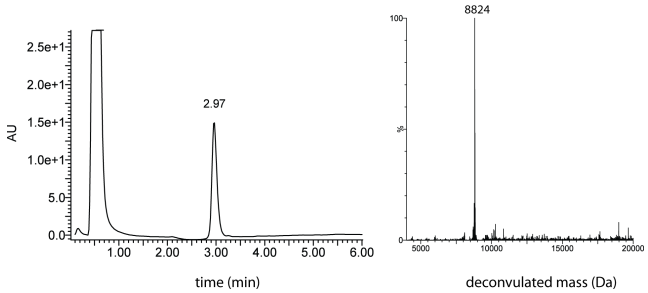


Figure 4. UbiQ-036: A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.8 mL/min, column T= 40°C. Gradient: 5-95% over 3.5 min.

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