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 light.

Productsheet

Background. UbiQ-L03 is a panel of 4 deubiquitylating 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 $\lambda_{Ex}/\lambda_{Ex}$= 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 Gly76 of ubiquitin and Rh110Gly results in an increase in fluorescence at $\lambda_{Ex}/\lambda_{Ex}$= 535/485 nm.[3]

- **TAMRA-Lys(Ub)-Gly (UbiQ-012)** is a fluorescence polarization assay reagent based on a 5-carboxytetramethylrhodamine (TAMRA $\lambda_{Ex}/\lambda_{Ex}$= 530/580 nm) modified Lys-Gly sequence that is linked to ubiquitine 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). In general, HEPES and Tris buffers are standard for DUB assays
- 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
- please note that certain DUBs react different to low or high NaCl concentrations.
- TCEP or DTT can be used as reducing agent for the DUB (see Wrigley et al. *Cell Biochem Biophys* **2011**, *60*, 99).

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.

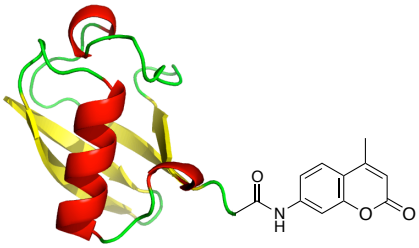
UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

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

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U

UbiQ
targeting the ubiquitin system



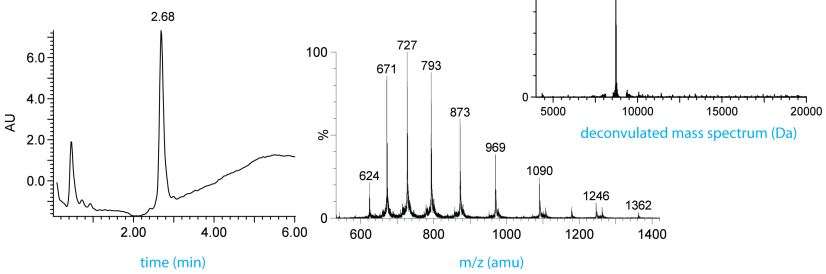
Ub-AMC *(human sequence, synthetic)*

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

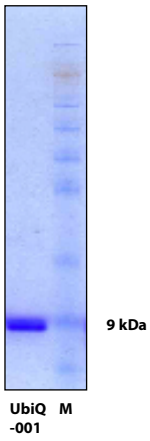
sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-**AMC**

A



B



A: 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 = 0.8 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5% ⇨ 95% over 3.5 min.
B: SDS-PAGE analysis. 12% SDS-PAGE gel. M= SeeBlue® Plus2 (Invitrogen). Coomassie Brilliant Blue staining.

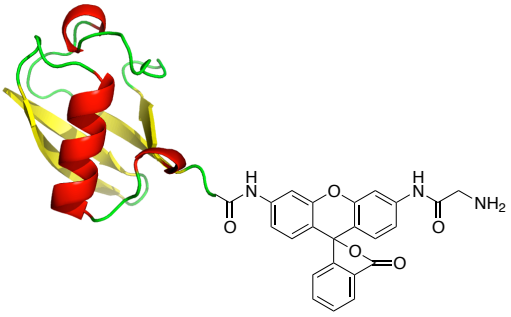
UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

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

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U

UbiQ
targeting the ubiquitin system



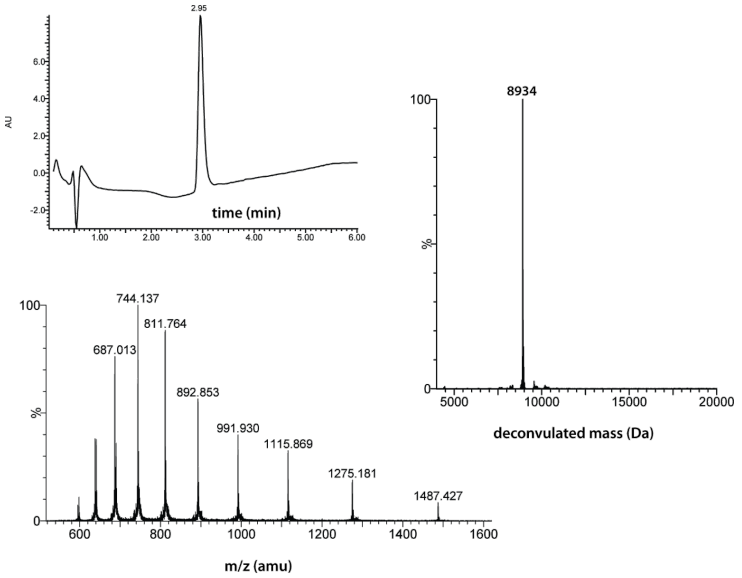
Ub-Rh110Gly *(human sequence, synthetic)*

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

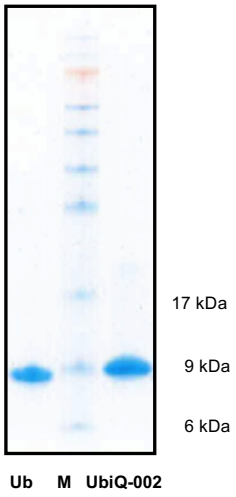
sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-**Rh110Gly**

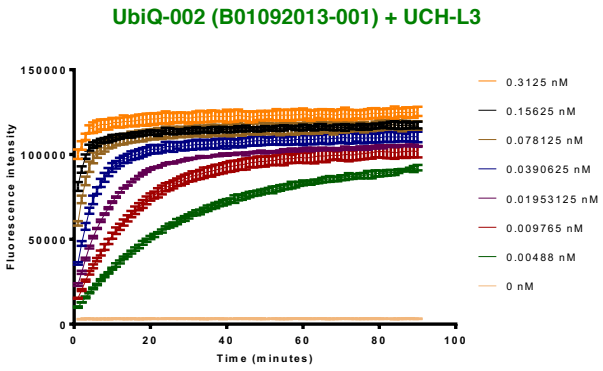
A



B



C



A: LC-MS analysis. 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. 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. **B: SDS-PAGE analysis.** 12% SDS gel, MES buffer. Coomassie Brilliant Blue staining. M= SeeBlue® Plus2 (Invitrogen). **C: Activity assay.** 100 nM UbiQ-002 (B01092013-001) with varying concentrations of UCH-L3 (5 - 313 pM).

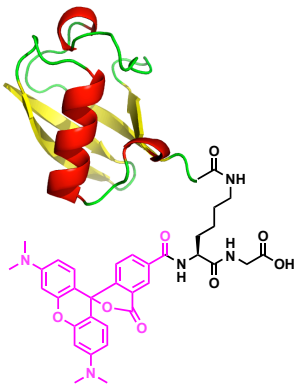
UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

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

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U

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

sequence

5-TAMRA-Lys(MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG)-Gly

Fluorescence polarization assay protocol

- FP assays were performed on a PerkinElmer Wallac EnVision 2010 Multilabel Reader with a 531 nm excitation filter and two 579 nm emission filters. Fluorescence intensities were measured in the S (parallel) and P (perpendicular) direction. FP values are given in mP (millipolarization) and calculated using formula (I)
- The confocal optics were adjusted with the average P and S values for TAMRA-Lys-Gly and the grating factor (G) was determined using a polarization value (L) for TAMRA of 50 mP using formula (II)
- The assays were performed in "non-binding surface flat bottom low flange" black 384-well plates (Corning) at room temperature in a buffer containing 20 mM Tris·HCl, pH 7.5, 5 mM DTT, 100 mM NaCl, 1 mg/mL 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonic acid (CHAPS) and 0.5 mg/mL bovine gamma globulin (BGG). Each well had a volume of 20 µL. Buffer and enzyme were predispensed and the reaction was started by the addition of substrate. Kinetic data was collected in intervals of 2.5 or 3 min. From the obtained polarization values (P_t) the amount of processed substrate (S_t) was calculated with formula (III); P_t is the polarization measured (in mP); P_{max} is the polarization of 100% unprocessed substrate (determined for every reagent at all used substrate concentrations); P_{min} is the polarization of 100% processed substrate; S_0 is the amount of substrate added to the reaction.
- From the obtained P_t values the values for initial velocities (v_i) were calculated, which were used to determine the Michaelis-Menten constants (K_m, V_{max} and k_{cat}) by fitting the data according to formula (IV) (where $k_{cat} = V_{max}/[E]$). All exp. data was processed using Ms Excel and Prism 4.03 (GraphPad Software, Inc).

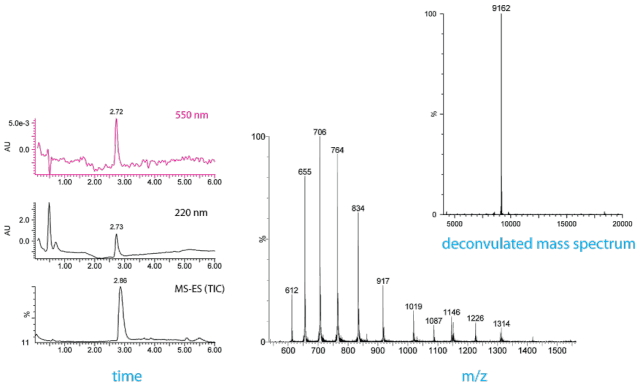
(I) $$Polarization\ (mP) = \frac{S-(G\cdot P)}{S+(G\cdot P)}\cdot 1000$$

(II) $$G = \frac{average\ S}{average\ P}\cdot\frac{1-(\frac{L}{1000})}{1+(\frac{L}{1000})}$$

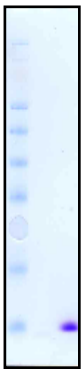
(III) $$S_t = S_0 - \left[S_0 \times \frac{P_t - P_{min}}{P_{max} - P_{min}}\right]$$

(IV) $$v_i = \frac{V_{max}\times S_0}{K_m + S_0}$$

A



B



A: 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. **B: SDS-PAGE analysis.** 12% SDS-PAGE gel. CBB staining. M= SeeBlue® Plus2 (Invitrogen)

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

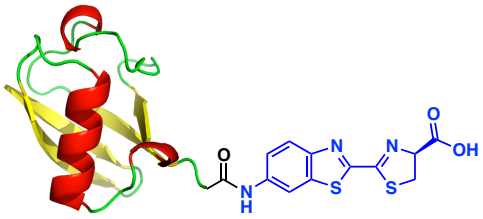
t +31 20 303 1970
e info@ubiqbio.com
i www.ubiqbio.com

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U

UbiQ
targeting the ubiquitin system

Ubiquitin-aminoluciferin *(human sequence, synthetic)*

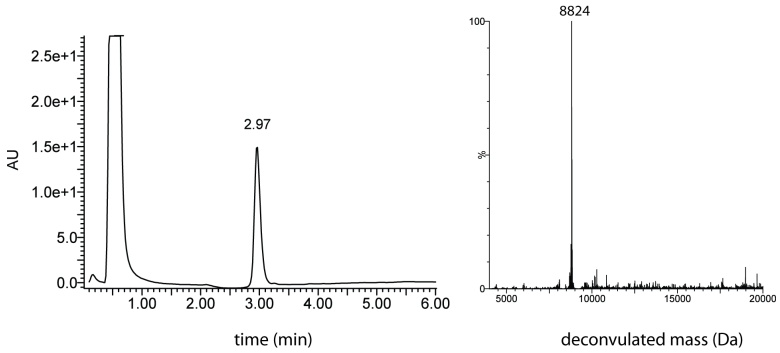
UbiQ code	:	UbiQ-036
Batch #	:	B01092013-001
Purity	:	≥95% by RP-HPLC
Amount	:	25 ug, lyophilized powder
Mol. Weight	:	8.82 kDa



sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-**(6-aminoluciferin)**

A



B

A: 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. **B: SDS-PAGE analysis.** 12% SDS-PAGE gel. Coomassie Brilliant Blue staining. M= SeeBlue® Plus2 (Invitrogen)

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

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

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U