UbiQ
targeting the ubiquitin system

(Biotin-Ahx-Ub)-(Cys-Ahx-Ub) K48 *(human sequence, synthetic)*

UbiQ code	:	UbiQ-119
Batch #	:	B01052015-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC.
Mol. Weight	:	17.64 kDa
Storage	:	upon arrival powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-119 is a native K48 linked di-Ub which is modified with a biotin tag on the distal Ub and an N-terminal cysteine on the proximal Ub.* An aminohexanoic acid (Ahx) linker is used to create extra space for efficient access of biotin binding entities and Cys reactive reagents. The Cys residue can be modified by two methods:

1) by thiol alkylation with thiol-reactive moieties (such as maleimides and iodoacetamides)
2) by native chemical ligation using activated esters (such as thioesters and NHS esters).

Ligation via method 2 retains the thiol group of the Cys residue which could then be used for attaching another label if desired. Overall, UbiQ-119 is designed to allow for the creation of various K48 diUb based conjugates which could serve for example as DUB assay reagents. This product is formed by chemical ligation.

Sequence

Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Cys-Ahx-Nle-QIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

* Met1 of the proximal Ub is replaced by norleucine (Nle)

Important: sample preparation

- **dissolve the powder in as little DMSO as possible (*e.g.* 20 mg/mL)**
- **add this DMSO stock slowly to milliQ (please note the order of addition)**
- **buffer the aq. solution as desired**
- **final stocks of e.g. 0.5 mg/mL will contain 2.5 vol% DMSO.**
- **buffer exchange using 3 kDa spin filters or dialysis membrane allows total removal of DMSO if desired.**

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

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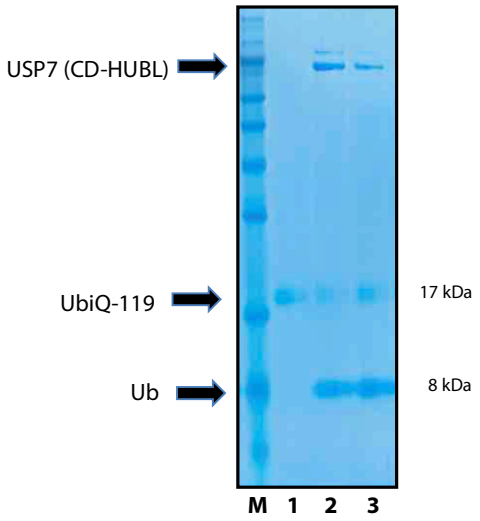
UbiQ-119 + USP7 (CD-HUBL).



1= UbiQ-119
2= 57 uM UbiQ-119 + 300 nM USP7 (CD-HUBL)
3= 57 uM UbiQ-119 + 30 nM USP7 (CD-HUBL)

Experimental conditons: a 20 mg/mL stock of UbiQ-119 in DMSO was dissolved in water to a concentration of 2 mg/mL. This was diluted to 1 mg/ml (57 uM) with 100 mM HEPES pH 8, 200 mM NaCl and 10 mM TCEP (resulting in 50 mM HEPES pH 8, 100 mM NaCl and 5 mM TCEP). Next, USP7 (catalytic+HUBL domain) was added and the reaxction incubated at 37°C for 1 hour. Samples were mixed with loading buffer, heated at 90°C for 10 min and analyzed by SDS-PAGE: 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer. Marker= SeeBlue Plus2 Pre-stained Standard (Invitrogen). CBB staining was performed with a *Coommassie G-250* solution.

Literature. (1) Faesen et al. *Chemistry & Biology* **2011,***18*, 1550. (2) Dikic et al. *Nature Reviews Molecular Cell Biology* **2010**, *10*, 659. (3) Licchesi et al. *Nature Structural & Molecular Biology* **2012**, *19*, 62. (4) El Oualid et al. *Angewandte Chemie Int. Ed.* **2010**, *49*, 10149.

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