UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-056
Batch #	:	B01112013-001
Amount	:	50 ug, lyophilized powder
Purity	:	>95% by RP-HPLC.
Mol. Weight	:	17.84 kDa
Storage	:	upon arrival, powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

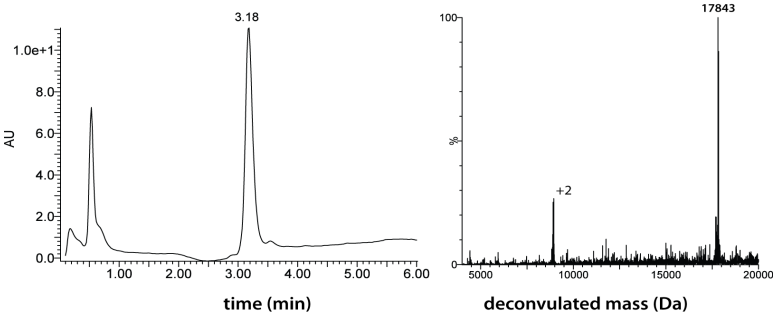
Background. UbiQ-056 is a native K48 linked diubiquitin which contains a biotin on the *N*-terminus of the proximal ubiquitin and a *N*-(2,4-dinitrophenyl) (DNP) group on the *N*-terminus of the distal ubiquitin. Both tags are separated from the N-terminus by an additional 6-aminohexanoic acid (Ahx) linker. UbiQ-056 can serve as substrate of proteases that cleave the isopeptide linkage between K48 linked chains.[1,2] This product is formed by chemical ligation.[3]

sequence

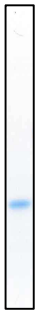
Biotin-Ahx- MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

DNP-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQKESTLHLVLRLRGG

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% B over 3½ min.
B: SDS-PAGE analysis UbiQ-056 (12% Bolt Bis-Tris, MES buffer), staining with CBB G-250.

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 3.5 kDa spin filters or dialysis membrane allows total removal of DMSO

Literature. (1) Horton et al. *Analytical Biochem* **2007**, *360*, 138. (2) Engels et al. *Analytical Biochem* **2009**, *390*, 85. (3) El Oualid et al. *Angew Chem Int Ed* **2010**, *49*, 10149.

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