UbiQ

targeting the ubiquitin system

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HTRF K48 linked diUbiquitin *(tag 1= biotin, tag 2= DNP) (human, synthetic)*

UbiQ code : UbiQ-056

Batch # : B01112013-001

Product Information

Amount : 50 ug lyophilized powder

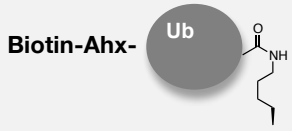
Purity : >90% by RP-HPLC and SDS-PAGE.

Mol. Weight : 17843 Da by MS (calc Mw 17840 Da)

Storage : powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Background. UbiQ-056 is a native K48 linked di-Ub which is equiped with a biotin on the N-terminus of the proximal ubiquitin and a dinitrophenyl group on the N-terminus of the distal ubiquitin (DNP= N-(2,4-dinitrophenyl)-6-aminohexanoic Acid). Both tags are separated from the N-terminus by an additional aminohexanoic acid (Ahx) linker. UbiQ-056 is designed to be used in an *Homogeneous Time-Resolved Fluorescence* (HTRF) assay as a substrate for proteases that cleave the isopeptide linkage between two ubiquitin molecules.[1,2] This product is formed by chemical ligation.[3]

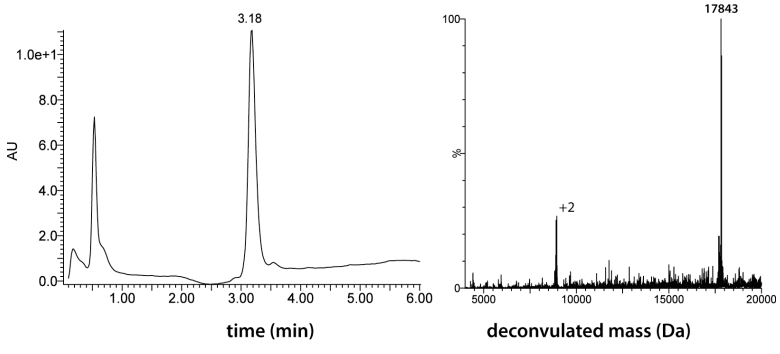
HTRF K48 linked diubiquitin



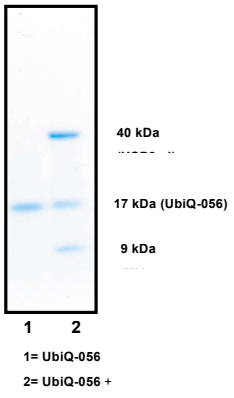
DNP-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQKESTLHLVLRLRGG

Important - Sample preparation. Dissolve the powder in a minimal amount of DMSO (e.g. 0.5 mg in 25 µL DMSO gives a stock of 1121 µM) and add this DMSO stock slowly to milliQ or the required buffer (please note the order of addition). Buffer exchange using 3 kDa spin filters (or dialysis membrane) can be used to remove the DMSO if this is required.

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 and cleavage of UbiQ-056 by USP8cd. **UbiQ-056** (3 ug in 10 uL, 18 uM) was incubated for 30 min at 37°C with USP8cd (3.7 uM) after which the reactions was stopped with sample buffer and boiling for 5 min. at 95°C. Proteins were separated by SDS-PAGE (12%, MES buffer) and stained with CBB G-250.

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

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