

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

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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-ubiquitin which is modified with an N-terminal biotin tag on the distal Ub and an N-terminal cysteine on the proximal Ub. A 6-aminohexanoic acid (Ahx) linker is used to create extra space for efficient access of biotin binding entities and Cys reactive reagents. The biotin can be used as conjugation site for labeled streptavidin or antibiotin antibodies. The Cys residue can be modified by two methods:

- by thiol alkylation with thiol-reactive moieties (such as maleimides and N-iodoacetamides)
- 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 (TR-FRET/FRET) DUB assay reagents.

Biotin-Ahx-MQIFVKTLTGKTI	LEVEPSDTIENVKAKIQDKEGIPP	DQQRLIFAGKQLEDGRTLSD	NIQKESTLHLVLRLRGG	
	EPSDTIENVKAKIQDKEGIPPDQQ	[

Figure 1. A: sequence; Met1 of the proximal Ub is replaced by the Met mimic, norleucine (NIe). B: SDS-PAGE analysis UbiQ-119: 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer, CBB staining.

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

Literature. (1) El Oualid et al. Angew Chem Int Ed 2010, 49, 10149. (2) Geurink et al. ChemBiochem 2016, 17, 816.

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