

UbiQ

targeting the ubiquitin system

Biotin-Ahx-Ub pSer 65 (human sequence, synthetic)

UbiQ code : UbiQ-091

Batch # : B01042016-001

Amount : 50 ug, lyophilized powder

Purity : $\geq 95\%$ by RP-HPLC and SDS-PAGE

Mol. Weight : 8.98 kDa

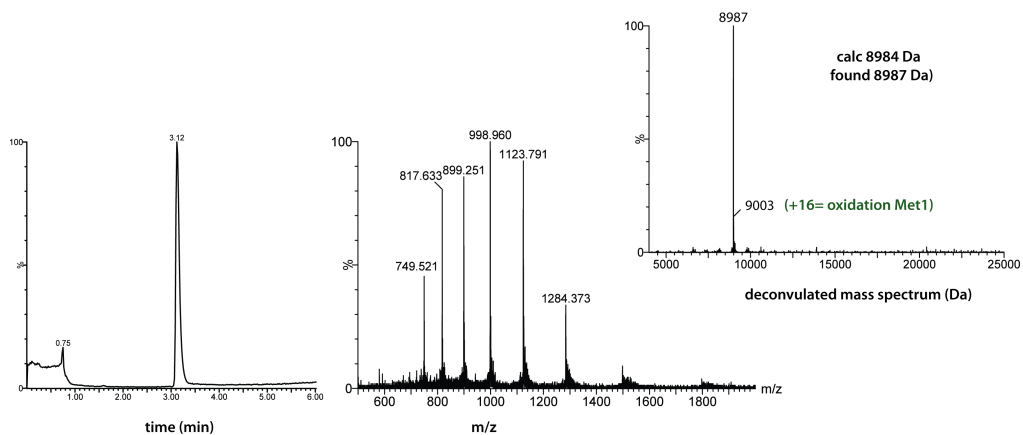
Storage : upon arrival powder at -20°C ; buffered solution at -80°C . Please avoid multiple freeze/thaw cycles.

Productsheet

Background - Biotin-Ahx-Ub pSer65 (UbiQ-091) is ubiquitin that is phosphorylated on Ser65, a ubiquitin variant which has been shown to activate Parkin E3 ligase mediated ubiquitination.¹⁻⁹ It is labeled on the *N*-terminus with biotin; an aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Ub protein for efficient access of biotin binding entities. It has been made by total chemical synthesis¹⁰ and is therefore well-defined in terms of biotin and pSer site and incorporation efficiency (100%).

Sequence

biotin-Ahx-MQIFVKTLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKE^SPTLHLVLRIRGG



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 . XBridge BEH300 C18 5 μm 4.6x100mm; column T = 40°C , flow = 0.8 mL/min. Gradient: 30–95% over 3.5 min.

Important: sample preparation

- **dissolve the powder in as little DMSO as possible (e.g. 40 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 1.25 vol% DMSO)**
- **buffer exchange using 3 kDa spin filters or dialysis membrane allows total removal of DMSO if desired.**

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Literature.

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- 3) Kondapalli *et al. Open Biol* **2012**, *2*, 120080.
- 4) Koyano *et al. Nature* **2014**, *510*, 162-166.
- 5) V. Sauve and K. Gehring *Cell Res* **2014**, *24*, 1025.
- 6) Spratt *et al. Nat Commun* **2013**, *4*, 1983.
- 7) Trempe *et al. Science* **2013**, *340*, 1451.
- 8) T. Wauer and D. Komander *EMBO J* **2013**, *32*, 2099-2112.
- 9) Yamamoto *et al. J Biol Chem* **2005**, *280*, 3390-3399.
- 10) El Oualid *et al. Angew Chem Int Ed* **2010**, *49*, 10149.