

## Biotin-Ahx-Ub pThr7 (human sequence, synthetic)

UbiQ code : UbiQ-092

Batch # : B01102014-001

Amount : 50 ug, lyophilized powder

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

Mol. Weight : 8.98 kDa

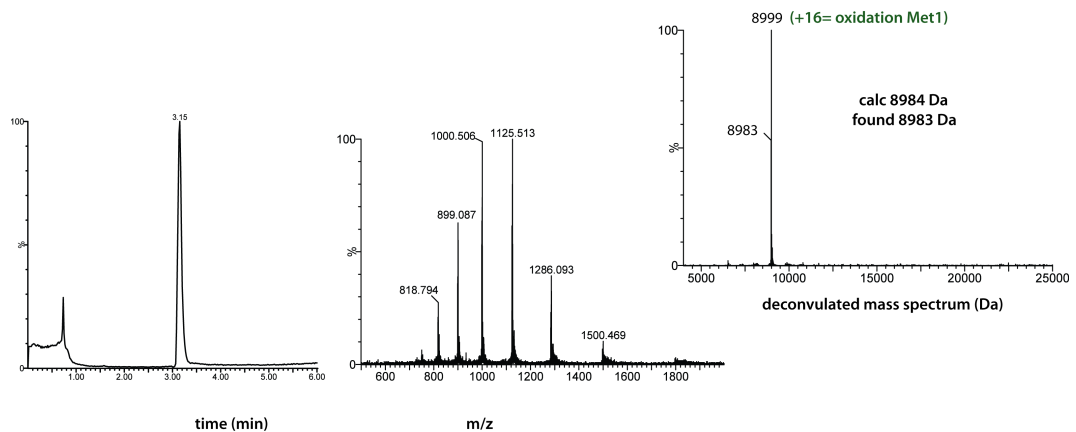
Storage : upon arrival powder at  $-20^{\circ}\text{C}$ ; buffered solution at  $-80^{\circ}\text{C}$ . Please avoid multiple freeze/thaw cycles.

## Productsheet

**Background.** Biotin-Ahx-Ub pThr7 (UbiQ-092) is a ubiquitin protein that is phosphorylated on Thr7. Phosphoproteomic studies have identified several phosphorylated sites in ubiquitin, among them pThr7.<sup>1-5</sup> 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 synthesis<sup>6</sup> and is therefore well-defined in terms of biotin and pThr site and incorporation efficiency (100%).

### Sequence

**biotin-Ahx-MQIFVK<sup>T</sup>P**LTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG



**LC-MS analysis.** Mobile phase A = 1%  $\text{CH}_3\text{CN}$ , 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in  $\text{CH}_3\text{CN}$ . XBridge BEH300 C18  $5\mu\text{m}$   $4.6 \times 100\text{mm}$ ; column T =  $40^{\circ}\text{C}$ , 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.



**Literature.**

- 1) Bennetzen et al. *Mol Cell Proteomics* **2010**, *9*, 1314.
- 2) Bian et al. *J Proteomics* **2014**, *96*, 253.
- 3) Kettenbach et al. *Sci Signal*, **2011**, *4*, rs5.
- 4) Sharma et al. *Cell Rep* **2014**, *8*, 1583.
- 5) Zhou et al. *J Proteome Res* **2013**, *12*, 260.
- 6) El Oualid et al. *Angew Chem Int Ed* **2010**, *49*, 10149.