

## Ub K33C (human sequence, recombinant)

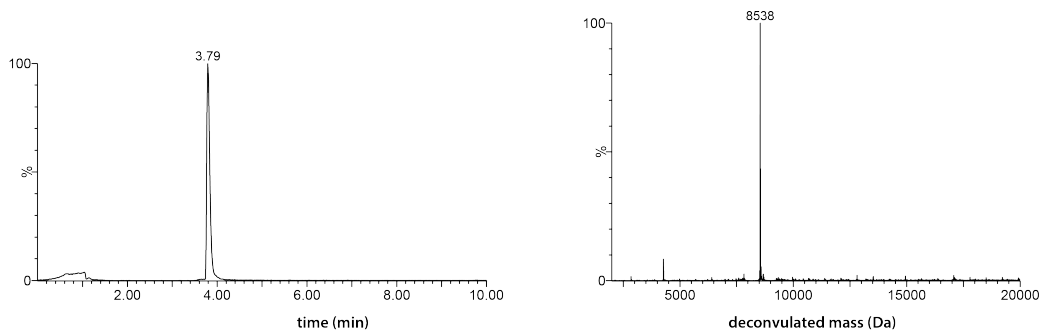
UbiQ code : UbiQ-178  
Batch # : B01092016-001  
Amount : 1.00 mg, lyophilized powder  
Purity :  $\geq 95\%$  by RP-HPLC  
Mol. Weight : 8.54 kDa  
Storage : upon arrival powder at  $-20^{\circ}\text{C}$ ; solution at  $-80^{\circ}\text{C}$ . Please avoid multiple freeze/thaw cycles.

## Productsheet

**Background.** Ub K33C (UbiQ-178) is based on the ubiquitin protein (Ub) in which lysine 33 has been mutated to a cysteine residue. Ub Lys-to-Cys mutants can be used to prepare well-defined Ub-chains<sup>1,2</sup> and site-selective functionalized Ub proteins (using thiol reactive moieties).

### Sequence

MQIFVKLTGTITLEVEPSDTIENVKAKIQDCEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG



**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  $5\mu\text{m}$   $4.6 \times 100\text{mm}$ ; flow rate = 0.8 mL/min, runtime = 10 min, column T =  $40^{\circ}\text{C}$ . Gradient: 30%  $\Rightarrow$  60% B over 6.5 min.

## Important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 20 - 40 mg/mL)
- add the DMSO stock slowly to milliQ (please note the order of addition)
- buffer the aq. solution as desired (e.g. 50 mM HEPES pH 8, 100 mM NaCl)
- for example, a final buffered stock of 0.5 mg/mL (59  $\mu\text{M}$ ) will contain 1.25 vol% DMSO when prepared from a 40 mg/mL DMSO stock.
- most DUBs and E1-E2-E3 enzymes tolerate DMSO concentrations up to 5 vol%
- if desired, the DMSO can be removed from the buffered stock by dialysis or 3 kDa spin-filters

**Literature.** (1) Raasi et al *Methods Mol Biol.* **2005**, *301*, 47. (2) Valkevich et al. *J Am Chem Soc.* **2012**, *134*, 6916.