

Ub K29C (human sequence, recombinant)

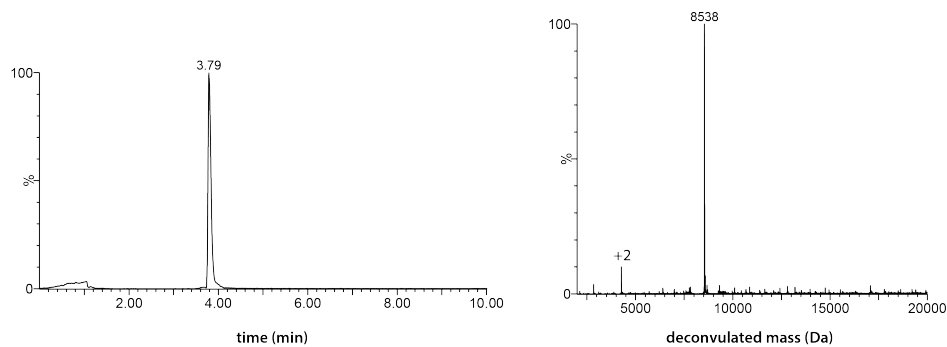
UbiQ code : UbiQ-177
Batch # : B01092016-001
Amount : 1.00 mg, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 8.54 kDa
Storage : upon arrival powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. Ub K29C (UbiQ-177) is based on the ubiquitin protein (Ub) in which lysine 29 has been mutated to a cysteine residue. Ub Lys-to-Cys mutants can be used to prepare well-defined Ub-chains^{1,2} and site-selective functionalized Ub proteins (using thiol reactive moieties).

Sequence

MQIFVKLTGTITLEVEPSDTIENVKACIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG



LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 30% ⇒ 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 µM) 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.