

UbiQ

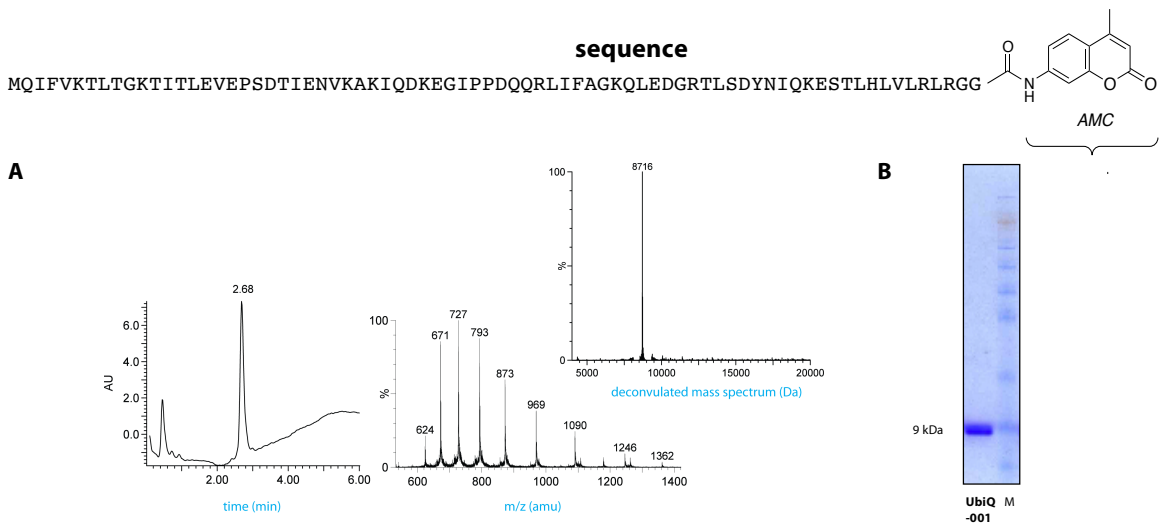
targeting the ubiquitin system

Ub-AMC (human sequence, synthetic)

UbiQ code : UbiQ-001
Batch # : B01012013-001
Amount : 100 ug, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 8.72 kDa
Storage : powder at -20°C; buffered solution at -80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. Ub-AMC is a quenched fluorogenic substrate for deubiquitinating enzymes.^{1,2} It is based on the C-terminal derivatization of ubiquitin with 7-amido-4-methylcoumarin (AMC).³ Release of AMC fluorescence by deubiquitinating enzymes is monitored using Ex380 nm and Em460 nm wavelengths, respectively.



A: 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. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.8 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5–95%B over 3.5 min. **B: SDS-PAGE analysis.** 12% SDS-PAGE gel. M= SeeBlue® Plus2 (Invitrogen). Coomassie Brilliant Blue

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
- final stocks of e.g. 0.5 mg/mL will contain 2.5 vol% DMSO.
- buffer exchange using 3 kDa spin filters or dialysis membrane allows total removal of DMSO if desired.

Literature. (1) Dang et al. *Biochemistry* **1998**, *37*, 1868. (2) Mason et al. *Biochemistry* **2004**, *43*, 6535. (3) El Oualid et al. *Angew Chem Int Ed* **2010**, *49*, 10149.