

UbiQ

targeting the ubiquitin system

Cy5-Ub-MMP (human sequence, synthetic)

UbiQ code : UbiQ-206

Batch # : B01042017-001

Amount : 50 ug, lyophilized powder

Purity : ≥95% by RP-HPLC

Mol. Weight : 9.1 kDa

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

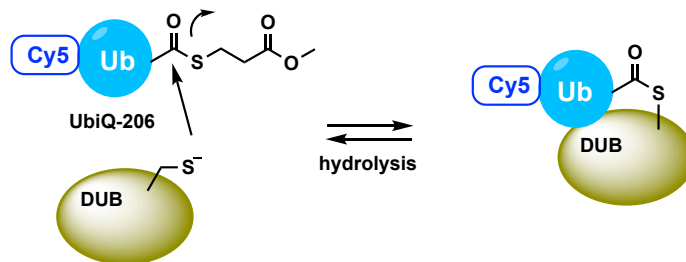


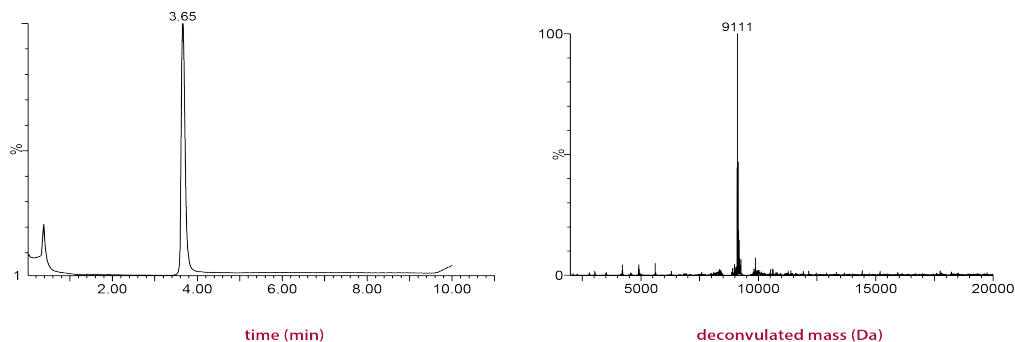
Figure 1.

Productsheet

Background. Cy5-Ub-MMP (**UbiQ-206**, Figure 1) is based on ubiquitin which is labeled on the *N*-terminus with a Cy5 dye (Cy5, exc 625 nm, emi 680 nm) and a *C*-terminal *methyl 3-mercaptopropionate* (MMP) thioester.² The reactive (electrophilic) *C*-terminal thioester can be used to trap proteins that react with the Ub *C*-terminus (e.g. deubiquitinating enzymes) via nucleophilic residues (like cysteine, Figure 1).¹ Cy5 dye allows detection by in-gel fluorescence, Met1 oxidation is eliminated by incorporation of norleucine as a well validated Met mimic.³

Sequence

Cy5-NIeQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPDPQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG-MMP



LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–60% over 6.5 min

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 as desired (with e.g. 1M HEPES to 50 mM HEPES): because the thioester is prone to hydrolysis at basic pH, we recommend to prepare probe stocks in buffer with a pH <7
- we also recommend to keep the pH as low as possible during labeling experiments
- as reducing agents we recommend to test TCEP and DTT
- a final buffered stock of for example 0.5 mg/mL contains 2.5 vol% DMSO; in general DMSO concentrations of up to 5 vol% are well tolerated by DUBs.
- if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).

Literature. (1) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (2) Song et al. *Prot Sci* **2009**, *18*, 2492. (3) Xu et al. *RSC Adv* **2016**, *6*, 47926. (2)