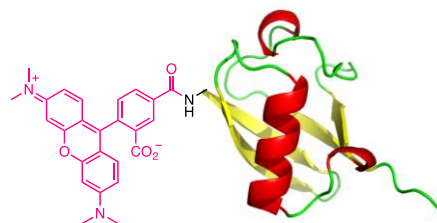


UbiQ

targeting the ubiquitin system



TAMRA-Ub (human sequence, synthetic)

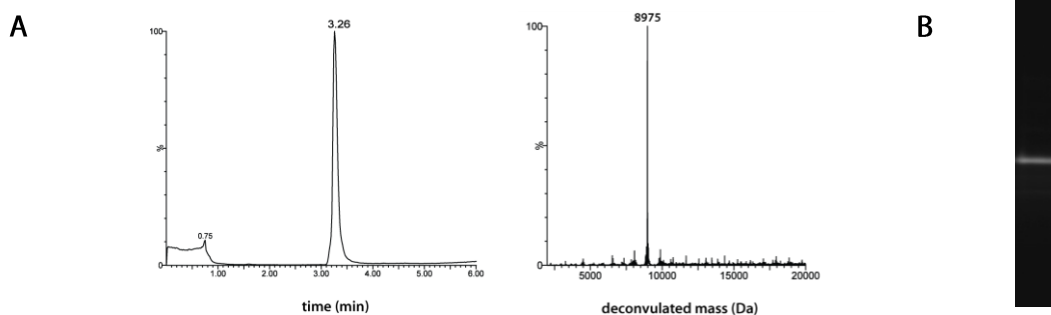
UbiQ code : UbiQ-003
Batch # : B01072014-001
Amount : 100 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC and SDS-PAGE
Mol. Weight : 8975 Da by MS (calc Mw 8977 Da)
Storage : upon arrival powder at -20°C ; buffered solution at -80°C . Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-003 is a Ub protein labeled on the N-terminus with the fluorescent dye TAMRA (5-tetramethylrhodamine, exc 550 nm, emi 590 nm). It has been prepared by total chemical synthesis¹ and allows detection of ubiquitylation by in-gel fluorescence.^{2,3} This direct and more sensitive read-out gives more distinct labeling patterns than immunoblotting and does not suffer from background labeling due to cross-reactivity (as seen sometimes with antibody based detection).

Sequence

TAMRA-MQIFVKLTGKITLEVPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG



A: LC-MS analysis. Mobile phase A = 1% CH_3CN , 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN . XBridge BEH300 C18 5 μm 4.6x100mm; column T = 40°C , flow = 0.8 mL/min. Gradient: 30–95% over 3.5 min. **B:** Fluorescent scan (550/590 nM) SDS-PAGE analysis UbiQ-003. 12% Bis-Tris, MES buffer.*

* Please note that during fluorescence scanning of SDS-PAGE gels with fluorescent Ub proteins, the appearance of higher mol. weight bands ("smearing") can be observed. We do not have (analytical) evidence these are actual contaminants present in the diUb sample but that they are aggregates formed during SDS-PAGE. We have also not witnessed any effect of this phenomenon on experiments performed with our diUb material. We advise to not overload the sample during SDS-PAGE analysis (<1 ug).

Important: sample preparation

- dissolve the powder in as little DMSO as possible (20 - 40 mg/mL)
- add the DMSO stock to milliQ (please note the order of addition) and mix
- buffer the aq. solution as desired (using 1M HEPES or 1M Tris for example)
- in general, DMSO concentrations up to 5 vol% are well tolerated by most enzymes.
- If required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).

Literature. (1) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149. (2) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (3) Smit et al. *J Biol Chem* **2013**, *288*, 31728.