

K58K(biotin) cRh110-Cys-Ubv^(USP7)-PA (synthetic, USP7 targeting Ub variant)

UbiQ code : UbiQ-314

Batch # : B01065021-001

Amount : 50 ug, lyophilized powder

Purity : >90%

Mol. Weight: 9.23 kDa

Storage : upon arrival, powder at -20°C; solution at -80°C. Please store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-314 is an activity-based probe that is based on a ubiquitin variant designed to selectively target USP7. It contains a C-terminal electrophilic propargyl amide (PA, also sometimes abbreviated as Prg).

- the PA group forms a covalent linkage with the active site Cys residue of USP7 that can be cleaved by acid treatment (5% aq. TFA), allowing proteomic analyses.
- the N-terminus is functionalised with a 5-carboxyrhodamine110 dye (cRh110, λ_{ex} = 480 nm; λ_{em} = 520 nm) allowing sensitive and fast (in-gel fluorescence) detection.
- a Cys residue is introduced between the dye and N-terminus for further modification with thiol-reactive tags.
- Ub variant is based on Ub with the following mutations: T7D, L8Y, I13R, E34L, Q40N, L69A, and L71A
- Lys58 contains a biotin tag which, besides detection and binding, also serves to increase USP7 selectivity by prohibiting binding of most USPs.

A

cRh110-C-MQIFVKDYTGKTRTLEVEPSDTIENVKAKIQDKLGIPDPNQRLIFAGKQLEDGRTLK(biotin)YNIQKESTLHAVARLRG-PA

B



C

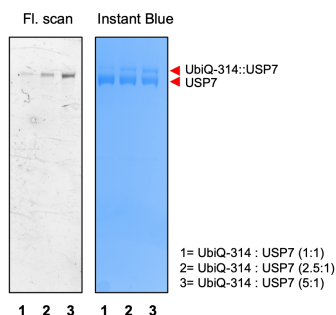


Figure 1. A: sequence UbiQ-314. B: SDS-PAGE analysis UbiQ-314. C: SDS-PAGE analysis UbiQ-314 + USP7. MES buffer, 12% Bolt Bis-Tris Plus gel (Lifetechnologies), fluorescent scanning (480/520 nm).

important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 25 – 40 mg/mL)
- add this DMSO stock slowly to milliQ (please note the order of addition)
- buffer as desired
- for experimental details please see references 1 and 2.

Literature. (1) Gjonaj et al. *Chem Comm* **2019**, 55, 5075. (2) Mandal et al. *Angew Chem Int Ed Engl* **2021**, 60, 7333. (3) Ekkebus et al. *J Am Chem Soc* **2013**, 135, 2867. (4) Sommer et al. *Bioorg Med Chem* **2013**, 21, 2511.