

UbiQ

targeting the ubiquitin system

(cRh110-Ub)-(TAMRA-Ub) K63 linked (human Ub sequence, synthetic)

UbiQ code : UbiQ-166

Batch # : B01042016-001

Amount : 25 ug

Purity : ≥95% by RP-HPLC and SDS-PAGE

Mol. Weight : 17.85 kDa

Storage : upon arrival store at -80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-166 is a recently developed native K63 linked di-Ub FRET reagent (Figure 1) which can be used as a substrate for proteases that cleave the isopeptide linkage between two ubiquitin proteins.¹ The distal Ub contains a 5-carboxyrhodamine110 (cRh110) dye on the N-terminus, the proximal Ub contains a 5-tetramethylrhodamine (TAMRA) dye on the N-terminus.

Sequence

cRh110-MQIFVKLTGKTITLEVPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG

TAMRA-MQIFVKLTGKTITLEVPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG

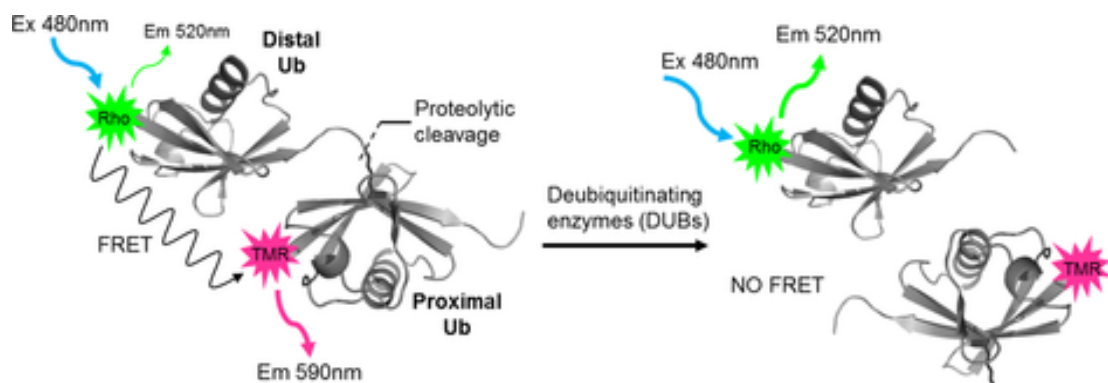


Figure 1. Principle of the FRET-based diUb cleavage assay. Upon cleavage of the diUb FRET pair by a DUB, the FRET signal is lost (from reference 1).

Experimental Procedures

- full experimental procedures can be found in the open-access reference 1: <http://onlinelibrary.wiley.com/doi/10.1002/cbic.201600017/full>
- assays can be performed in “non binding surface flat bottom low flange” black 384- well plates (Corning) at room temperature in a buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.6, 2.0 mM DTT, 1 mg/mL 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonic acid (CHAPS) and 0.5 mg/mL γ -globulins from bovine blood (BGG).

Literature. (1) Geurink et al. *ChemBiochem* **2016**, *17*, doi/10.1002/cbic.201600017