

# UbiQ

targeting the ubiquitin system

## Ub-PTEN(5-21)-FP

UbiQ code : UbiQ-030

Batch # : B10102013-001

Amount : 25 ug, lyophilized powder

Purity :  $\geq 95\%$  by RP-HPLC

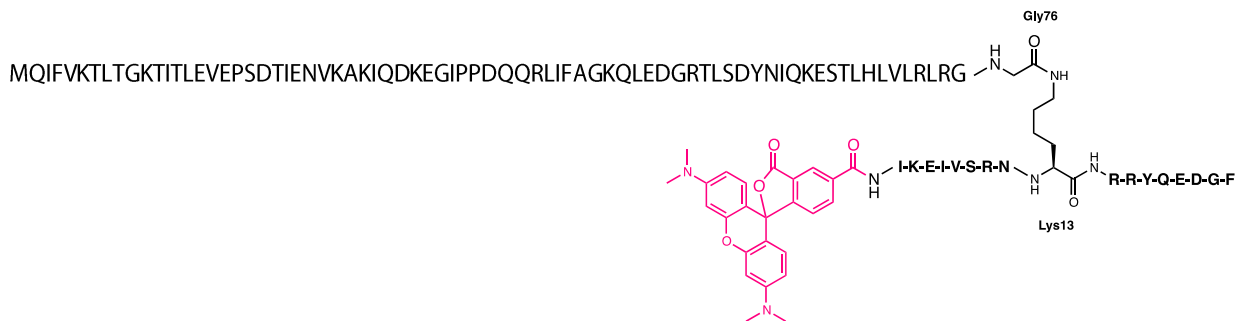
Mol. Weight : 11.104 kDa by MS (calculated 11.097 Da)

Storage :  $-20^{\circ}\text{C}$ ; buffered solution at  $-80^{\circ}\text{C}$ . Please avoid multiple freeze/thaw cycles.

## Productsheet

**Background.** UbiQ-030 is a fluorescence polarization HTS reagent based on amino acid sequence 5 – 21 of PTEN, a tumor suppressor phosphatase which is monoubiquitinated on Lys13 and Lys289. Monoubiquitination of these sites is important for regulation of PTEN mediated tumor suppression and its nuclear import. The peptide is modified on the N-terminus with 5-carboxytetramethylrhodamine (5-TAMRA) and conjugated at Lys13 to Ub via a native isopeptide bond.

### Sequence



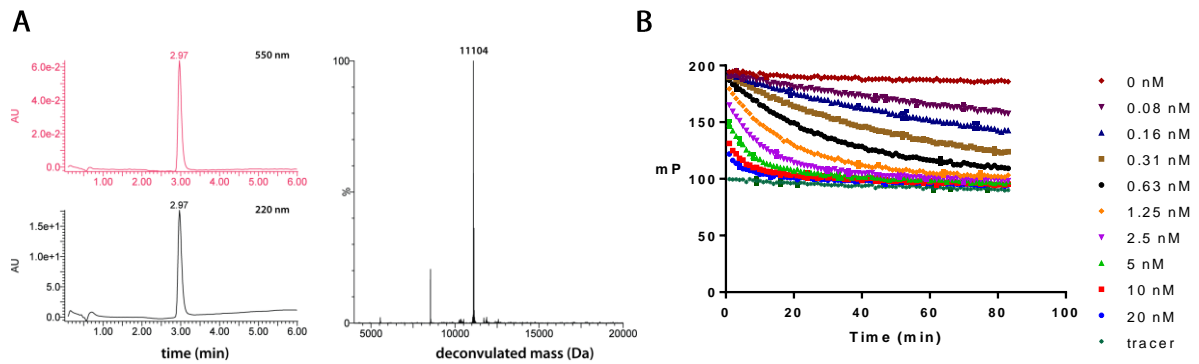
### Important: sample preparation.

- dissolve the powder first in DMSO and add this DMSO stock slowly to milliQ (please note the order of addition).
- next buffer with e.g. 1M HEPES to 50 mM HEPES. In general HEPES and Tris buffers are standard for DUB assays. Please note that certain DUBs react different to low or high NaCl concentrations.
- 2 - 5 mM TCEP or DTT can be used as reducing agent for the DUB.<sup>3</sup>
- 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).
- **Example of standard assay stock preparation:**
  - prepare a 11.1 mg/mL (1 mM) stock in DMSO
  - dilute 10 $\times$  into milliQ (1.1 mg/mL = 100  $\mu\text{M}$  stock in milliQ/10vol% DMSO)
  - buffer with 1M HEPES or 1M Tris (NaCl is optional)
  - this 100  $\mu\text{M}$  stock can be diluted to the standard 100 nM assay concentration (=1000 $\times$  dilution)
  - for assays with concentrations in the  $\mu\text{M}$  range, one can prepare more concentrated DMSO stocks and subsequently also more concentrated buffered stocks.

For assay details, please see the open-access publication: Geurink et al. *ChemBiochem*, 2012, 13, 293.

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**A: LC-MS analysis.** Mobile phase A = 1% CH<sub>3</sub>CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH<sub>3</sub>CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 μM; flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5%⇒95% over 3.5 min. **B: FP assay** with 100 nM UbiQ-030 B10102013-001 and different concentrations of USP7.

**Literature.** (1) Tirat, A. et al. *Anal. Biochem.* **2005**, *343*, 244-255. (2) Huang et al. *Methods in Molecular Biology* **2009**, *565*, 127. (3) Levine et al. *Anal. Biochem.* **1997**, *247*, 83. (4) Geurink and El Oualid et al. *ChemBiochem*, **2012**, *13*, 293.