Ubiquitin-Rhodamine 110

Ubiquitin substrate

Cat. No. **60-0117-050**
Lot. No. **30392**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

In addition to fusion proteins, ubiquitin derivatives conjugated with a fluorophore have been reported as substrates for biochemical DUB assays. Ubiquitin-Rhodamine 110 (Ub-Rho110-G) is a fluorogenic rhodamine-based substrate. While the disubstituted rhodamine moiety in Ub-Rho110-G is essentially non-fluorescent, cleavage results in a mono-substituted rhodamine, Rho110-G, which exhibits intense fluorescence when excited at 485 nm (Hassiepen *et al.*, 2007). The rhodamine fluorophore exhibits optical properties more appropriate – than Ubiquitin-AMC – for compound screening and profiling. The risk of artifacts in screens due to autofluorescence of compounds is substantially reduced as the rhodamine 110 fluorophore has excitation and emission wavelengths of 485nm and 535nm respectively (Hassiepen *et al.*, 2007).

References:

Hassiepen U, Eidhoff U, Meder G, Bulber JF, Hein A, Bodendorf U, *et al.* (2007) A sensitive fluorescence intensity assay for deubiquitinating proteases using ubiquitin-rhodamine110-glycine as substrate. *Anal Biochem* **371**, 201-207.

Physical Characteristics

Species: human

Source: synthetic

Quantity: 50 µg

Concentration: 2 mg/ml

Formulation: DMSO

Molecular Weight: 8.93 kDa

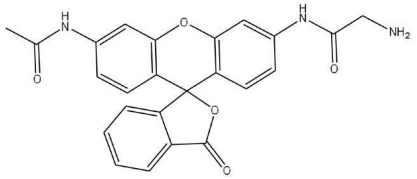
Purity: >95% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:

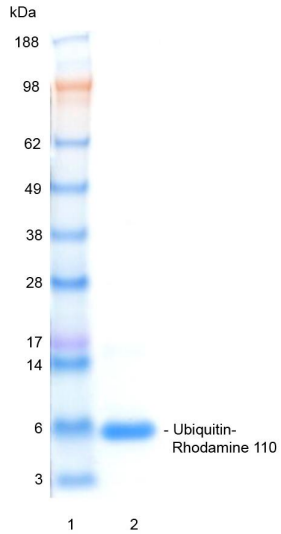
MQIFVKTLTGKTITLEVEPSDTIEN
VKAKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLRGG

Ubiquitin (amino acid residues 1-76)
C-terminally tagged with Rhodamine 110
Accession number: P62987



Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Ubiquitin-Rhodamine 110



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The activity of Ubiquitin-Rhodamine 110 was validated by determining the increase in fluorescence at 535nm (Excitation 485nm) measured as a result of the enzyme catalysed cleavage at the amide bond between the C-terminal Glycine and Rhodamine, generating Ubiquitin and dequenched Rhodamine 110-Glycine. UCHL3 (deubiquitylase) was incubated with Ubiquitin-Rhodamine 110 and the fluorescence was measured at four time points (0min, 30min, 60min and 90min).

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Lot-specific COA version tracker: v1.0.0