Ubiquitin-Rhodamine 110

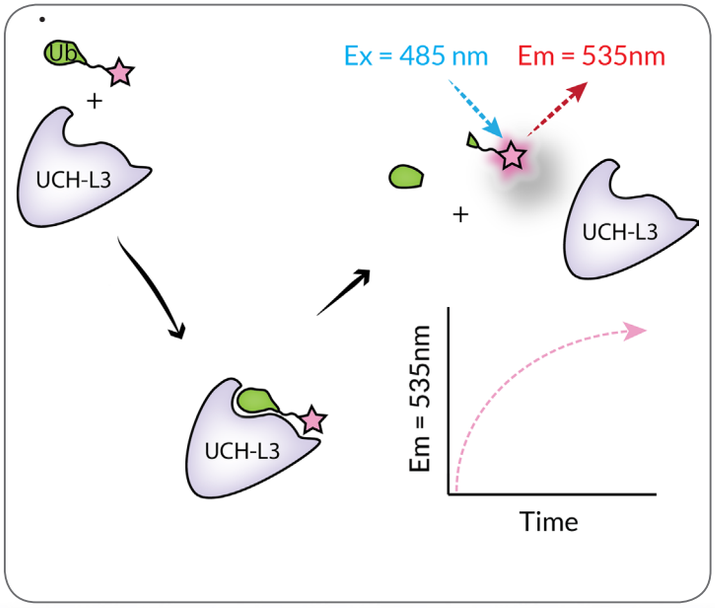
Cat. No. SBB-PS0001
Lot. No. 163060001

South Bay Bío

Ubiquitin-Rhodamine 110

Ubiquitin is a 76 amino acid post-translational modifier expressed throughout all tissues in eukaryotic organisms. The many roles of ubiquitin modification include proteasomal degradation, signal transduction, inflammatory response, and DNA damage repair. Ubiquitin modification occurs through a pyramidal cascade of an E1 activating enzyme, E2 conjugating enzymes, and an E3 ubiquitin ligases. This enzymatic cascade results in modification of a 3-amine of a lysine reside on a substrate protein. Substrates may either be mono or poly-ubiquitinated by M1, K6, 11, 27, 29, 33, 48 or 63 linkages. Removal of ubiquitin from a substrate protein occurs via deconjugating enzymes, of which there are nearly 100 known enzymes with various specificities.

This product consists of a full-length human, mature ubiquitin polypeptide (amino acids 1-76) conjugated on its c-terminus to a quenched Rhodamine 110 dye. Hydrolysis of the conjugate results in fluorescence observable by excitation at 485nm and emission at 535nm, which substantially reduces the risk of autofluorescence of compounds in screenings (Hassapien et al., 2007). Typical working range is 50-500nM.



Product Information

Quantity: 50µg **Molecular Weight:** 8.93 kDa

Concentration: 300µM, 2.7 mg/mL

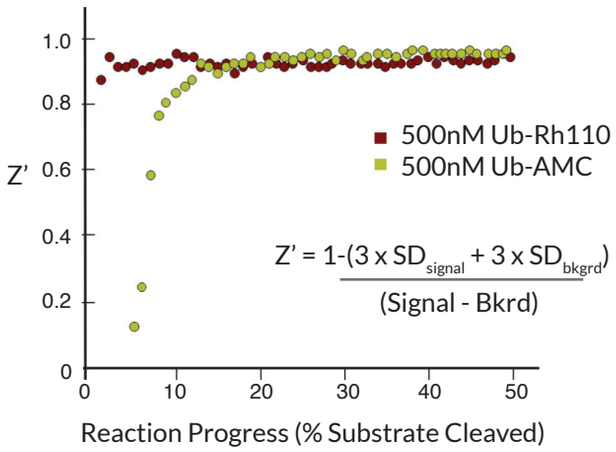
Purity: >99% by LCMS

Excitation/Emission = 485nm/535nm

Storage Buffer: 50mM MES, pH 6.0

Store at -80°C. Avoid multiple freeze thaw cycles.

Quality Control and Performance Data



Robustness of Rhodamine110 vs 7-amino-4-methylcoumarin (AMC) substrates in an HTS format.

Fluorescent substrates (500 nM Ub-Rh110/Ub-AMC) were incubated with and without 5 pM UcHL3 in a 384 well plate (n = 16), and progress curves were normalized to the maximum fluorescence signal to produce "% reaction progress". The Z' value, a statistical parameter widely used in the evaluation of screening assays, was calculated at each time-point.

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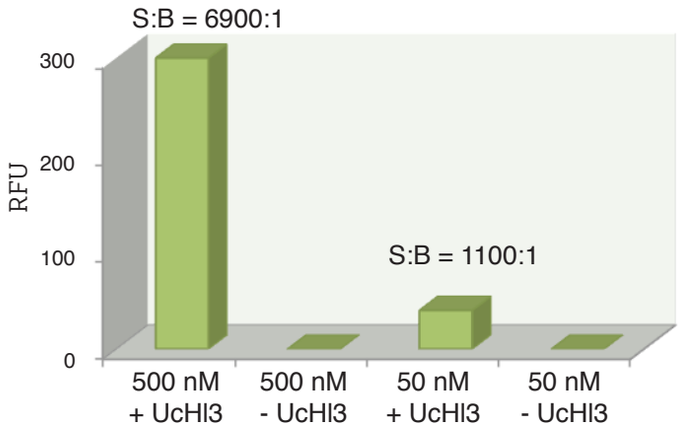
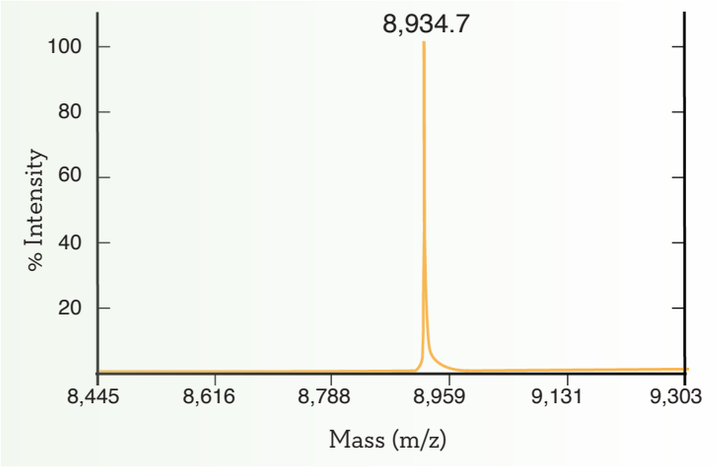
Quality Control and Performance Data

Ubiquitin Rh110 Signal : Background



Signal to Background.
The signal to background ratio was determined by 100% hydrolysis of either 50nM or 500nM Ubiquitin-Rhodamine 110 to liberate the quenched conjugate. Assay Buffer: 50mM HEPES pH7.5, 100mM NaCl, 1mM TCEP, 0.1mg/ml BSA.

Mass Spectrometry Data



References

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

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