Ubiquitin-AMC

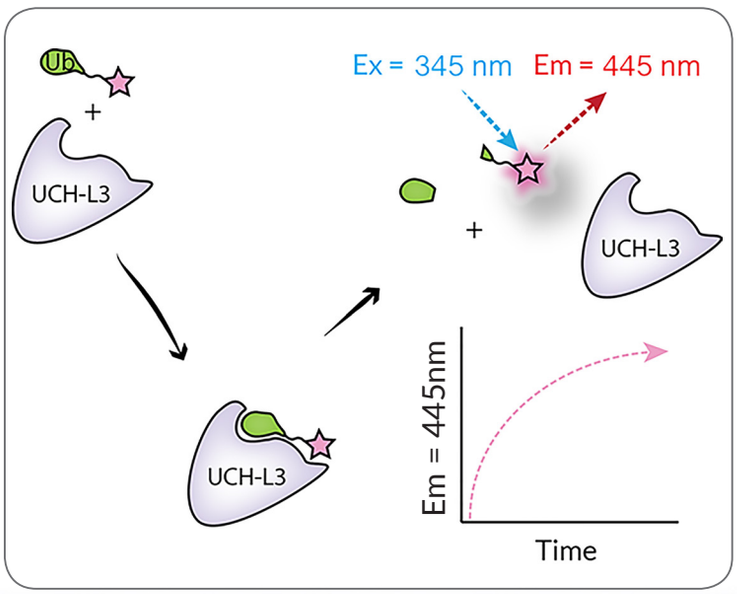
Cat. No. SBB-PS0043
Lot. No. 172440043

South Bay Bío

Ubiquitin-AMC

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) recombinantly expressed in *E.coli*, conjugated on its c-terminus to a quenched AMC dye. Hydrolysis of the conjugate results in fluorescence observable by excitation at 345nm and emission at 445nm, which substantially reduces the risk of autofluorescence of compounds in screenings (Dang et al., 1998). Typical working range is 50-500nM.



Product Information

Quantity: 50µg **Molecular Weight:** 8.7 kDa

Concentration: 300µM, 2.65 mg/mL

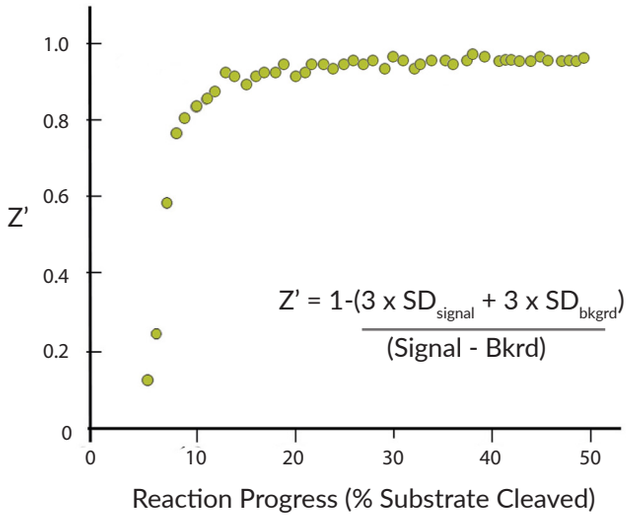
Purity: >99% by LCMS

Excitation/Emission = 345nm/445nm

Storage Buffer: 50mM MES, pH 6.0

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

Quality Control and Performance Data



Robustness of Ubiquitin-AMC substrates in an HTS format .
500 nM Ub-AMC was incubated with and without 5pM UcHL3 in a 384 well plate (n = 16), and progress curves were normalized to the maximum fluorescence signal to produce, i.e. the "% 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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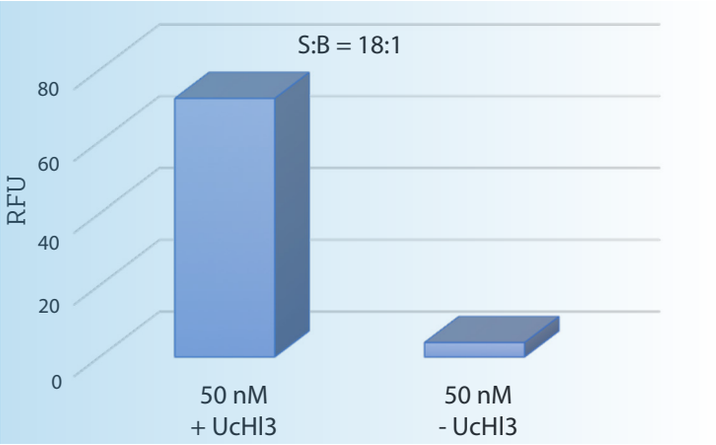
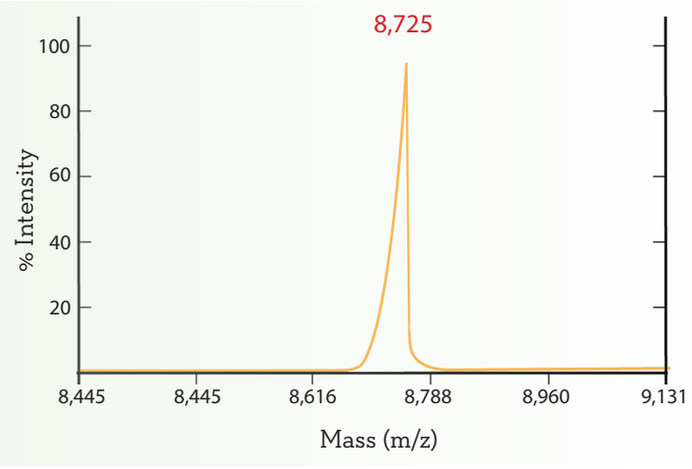
Quality Control and Performance Data

Ubiquitin AMC Signal : Background



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

Mass Spectrometry Data



References

1) Dang, L.C., Melandri, F.D. and Stein, R.L. Kinetic and mechanistic studies on the hydrolysis of ubiquitin C-terminal 7-amido-4-methylcoumarin by deubiquitinating enzymes. Biochemistry, 37, 1868-1879 (1998)

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