

Recombinant Full Length Human Ubiquitin Protein, Rhodamine 110 Labeled

Cat. No. Ubiquitin-07HFL **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This product consists of a full-length, mature ubiquitin polypeptide (amino acids 1-76) recombinantly expressed in E. coli, 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.
Species	Human
Source	E.coli
ProteinLength	1-76aa
Description	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 ϵ -amine of a lysine residue 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.
Conjugation/Label	Rhodamine 110

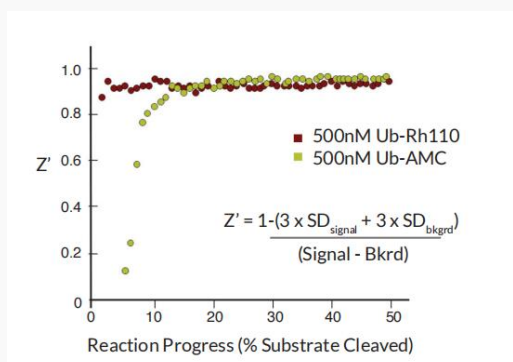
 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

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Molecular Mass	8.9 kDa
Purity	>99% by LCMS
Usage	Typical working range: 50-500nM
Notes	For Research Use Only, Not For Use In Humans.
Storage	Store at -80 centigrade. Avoid multiple freeze thaw cycles.
Storage Buffer	50mM MES, pH 6.0
Concentration	300μM, 2.7 mg/mL
Detection Method	Fluorescence
Substrate Properties	Protein-Based Substrate
Excitation/Emission (nm)	The Excitation and Emission of this substrate is 485nm and 535nm respectively.

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

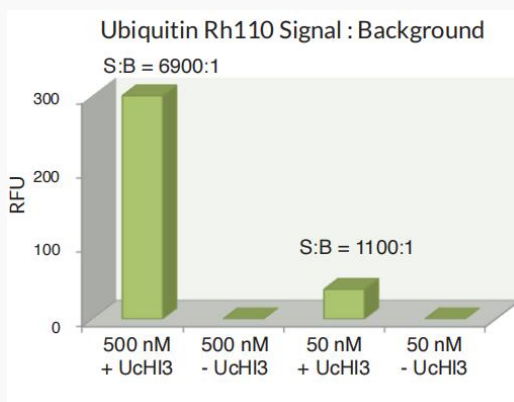
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without 5 pM UchL3 in a 384 well plate (n = 16), and progress curves were normalized to the maximum fluorescence signal to produce "% reaction progress". The Z' value, a statistical parameter widely used in the evaluation of screening assays, was calculated at each time-point.

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

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