

# ISG15-Rhodamine 110

Cat. No. SBB-PS0002  
Lot. No. 163060002

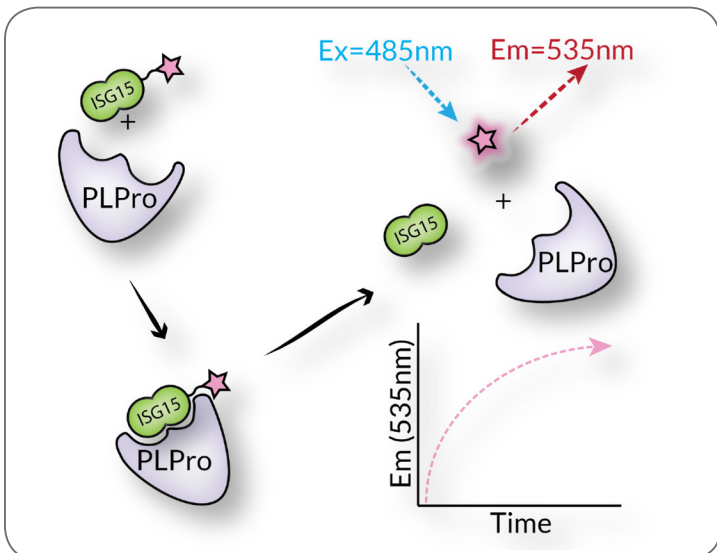


# South Bay Bio

## ISG15-Rhodamine 110

ISG15 (Interferon Stimulated Gene, 15kDa) is a Ubiquitin like modifier which initiates innate immune response by activating RIG-I signaling, stimulating NK-cell proliferation, inhibiting viral budding, and acting as an IFN $\gamma$ -inducing cytokine. ISG15 contains two tandem ubiquitin homology domains and is cross-reactive with  $\alpha$ -Ubiquitin antibodies. Conjugation of ISG15 to a substrate protein occurs through a Ubiquitin like cascade via an E1 (UBE1L), E2 (UbcH8/UBE2L6) and E3 (not yet discovered.) Deconjugation normally occurs via UBP43 (USP18), however there are several viral proteases that are able to hydrolyze ISG15 conjugates in order to evade immune response. These include the OTU-containing protease of Crimean-Congo Hemorrhagic Fever virus, and the Papain-Like Protease (PLPro) of the SARS coronavirus.

This product consists of a full-length, mature ISG15 polypeptide (amino acids 2-157) 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, which substantially reduces the risk of autofluorescence of compounds in screenings (Hassapien et al., 2007). Typical working range is 50-500nM.



## Product Information

**Quantity:** 50 $\mu$ g **Molecular Weight:** 17.6 kDa

**Concentration:** 60  $\mu$ M, 1.05 mg/mL

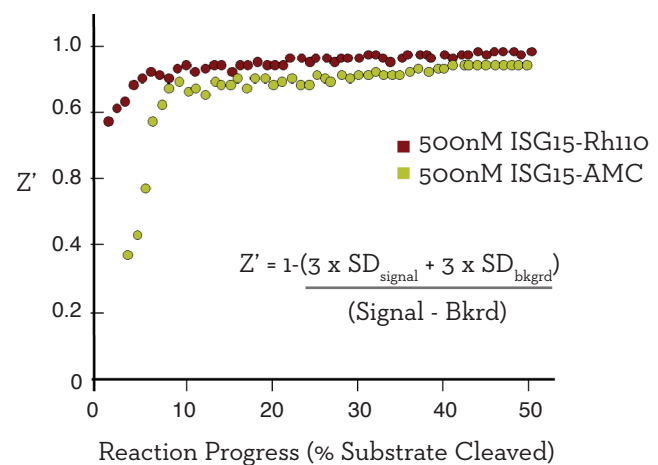
**Purity:** >97% by LCMS

**Excitation/Emission** = 485nm/535nm

**Storage Buffer:** 50mM MES pH 6.0, 100mM NaCl, 10% Glycerol

Store at -80 $^{\circ}$ C. Avoid multiple freeze thaw cycles.

## Quality Control and Performance Data



### Robustness of Rhodamine 110 vs 7-amino-4-methylcoumarin (AMC) substrates in an HTS format.

Fluorescent substrates (500 nM ISG15-Rh110/ISG15-AMC) were incubated with and without 100 pM PLpro (SARS Papain-Like-Protease) 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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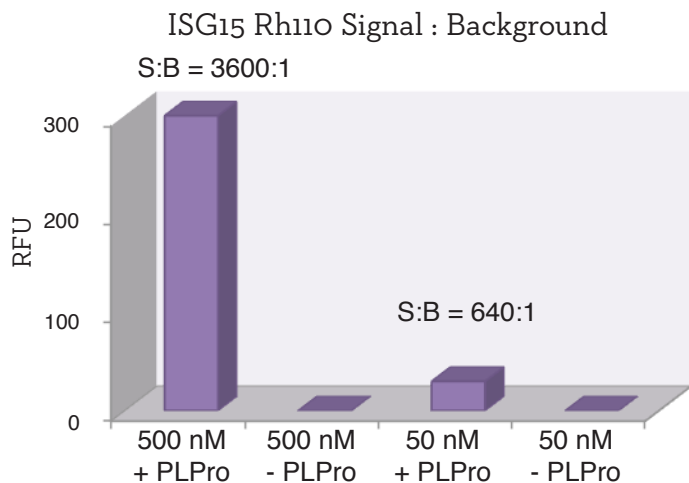
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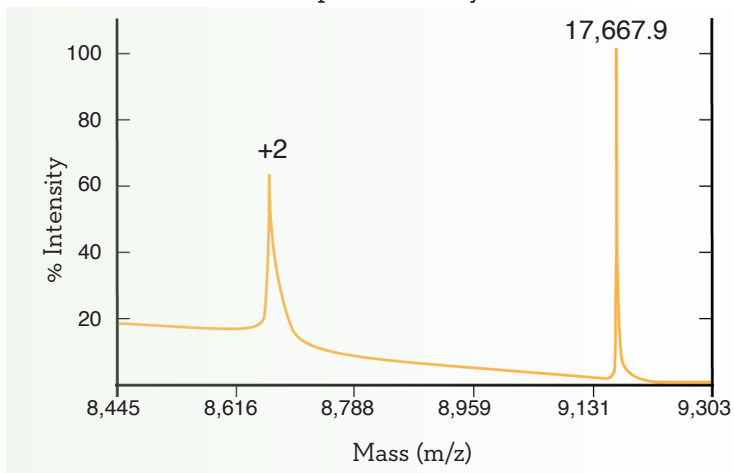
## Quality Control and Performance Data



### Signal to Background.

The signal to background ratio was determined by 100% hydrolysis of either 50nM or 500nM ISG15-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) Basters, et al. (2012) High yield expression of catalytically active USP18 (UBP43) using a Trigger Factor fusion system. BMC Biotechnology 12:56.

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