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32-190039: ISG15 (human) (Rhodamine 110)

Reactivity : Human
Uniprot ID : P05161
Alternative Name : ISG15 Ubiquitin-like Modifier; G1P2; IFI15; IP17; UCRP

Description

Source: E. coli ,
MW ~18kDa ,

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 IFNγ-inducing cytokine. ISG15 contains two tandem ubiquitin homology domains and is cross-reactive with α-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 nairovirus and the Papain-Like Protease (PLPro) of the SARS coronavirus.

Product Info

Amount : 50 µg
Purification : >=97% (LCMS)
Content : Liquid. In 50mM MES pH 6.0, 100mM sodium chloride, 10% glycerol.
Storage condition : Short Term Storage -80°C Long Term Storage -80°C Handling Advice Aliquot to avoid freeze/thaw cycles. Use/Stability Stable for at least 1 year after receipt when stored at -80°C.
Amino Acid : Human ISG15 (aa1-157) (Accession Nr. P05161) conjugated at the C-terminus to a quenched Rhodamine 110 dye.

Application Note

Protein-based substrate. Typical working concentration range is 50-500nM. Hydrolysis of the conjugate results in fluorescence observable by excitation at 485nm and emission at 535nm.

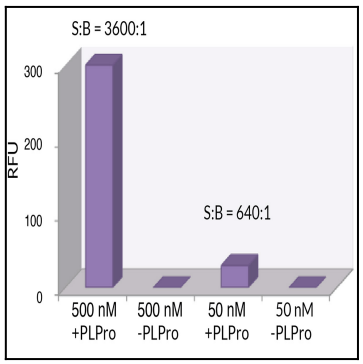

Figure 1: Signal to Background. The signal to background ratio was determined by 100% hydrolysis of either 50nM or 500nM ISG15-Rhodamine 110 to liberate the quenched conjugate. Assay Buffer: 50mM HEPES pH7.5, 100mM NaCl, 1mM TCEP, 0.1mg/ml BSA.

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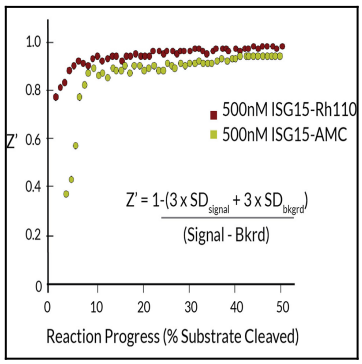

Figure 2: Robustness of Rhodamine 110 vs. 7-amino-4- methylcoumarin (AMC) substrates. Fluorescent substrates (500nM ISG15-Rh110/ ISG15-AMC) were incubated with and without 100pM PLpro (SARS Papain-Like-Protease) in a 384 well plate (n=16), and progress curves were normalized to the maximum fluorescence signal to produce â€œ% reaction progressâ€. The Zâ€™ value, a statistical parameter widely used in the evaluation of screening assays, was calculated at each time-point.