

DESCRIPTION

Source *E. coli*-derived human ISG15/UCRP protein
Met1 - Cys157 (C78S, G157C) with a C-terminal 6-His tag; C-terminal AMC derivatized protein
Accession # P05161.5

Predicted Molecular Mass 18 kDa

SPECIFICATIONS

Activity This fluorogenic substrate for ISG15 hydrolases is based on the carboxy-terminus derivatization of ISG15 with 7-amido-4-methylcoumarin (AMC). Release of AMC fluorescence can be monitored with an excitation wavelength of 380 nm and an emission wavelength of 460 nm. Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human ISG15 AMC concentration of 0.1-1 µM.

Formulation Supplied as a solution in HEPES, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Interferon-stimulated Gene 15 (ISG15), also known as Ubiquitin Cross-reacting Protein (UCRP), is a Ubiquitin-like protein that is covalently coupled to target proteins in a process termed ISGylation. It is a 165 amino acid (aa) polypeptide with a predicted molecular weight of 18 kDa. ISG15/UCRP exhibits 66% aa sequence identity with its mouse ortholog. Structurally, ISG15/UCRP consists of two tandem Ubiquitin-like domains that share a similar 3-dimensional structure with Ubiquitin and other Ubiquitin-like modifiers including NEDD8 and SUMO1. Modification of targets by ISG15/UCRP occurs in a stepwise enzymatic process similar to that of Ubiquitin. Enzymes regulating ISGylation include the activating (E1) enzyme UBE1L, the conjugating (E2) enzyme UbcH8, and ligases (E3) such as EFP/TRIM25 and HERC5 (1-4). Removal of ISG15/UCRP is catalyzed by the deconjugating enzyme UBP43/USP18 (5). Functionally, ISG15/UCRP has putative roles in the immune response and tumorigenesis. This is reflected by intracellular ISG15/UCRP targets that include Cyclin D1, tumor suppressor p63, IRF3, and a range of viral proteins (6-8). It is induced by type 1 interferons and microbial infection, and knockout mice exhibit an increased sensitivity to infection by some viruses (6). ISG15/UCRP can also be secreted by cells of the immune system and may act in a cytokine-like manner (9). For instance, it is produced by human granulocytes in response to mycobacterium exposure, and natural killer cells and T cells respond to extracellular ISG15/UCRP with IFN-gamma production (10). Further supporting a role in immune function, ISG15/UCRP mutations are associated with MSMD, an inherited disorder characterized by increased susceptibility to mycobacterial infection (10). ISG15 AMC is useful for studying enzymes (such as UBP43 and Papain-Like Protease from SARS coronavirus) when detection sensitivity or continuous monitoring of activity is essential.

References:

1. Yuan, W. & R.M. Krug (2001) EMBO J. **20**:362.
2. Zhao, C. *et al.* (2004) Proc. Natl. Acad. Sci. USA **101**:7578.
3. Zou, W. & D.E. Zhang (2006) J. Biol. Chem. **281**:3989.
4. Wong, J.J. *et al.* (2006) Proc. Natl. Acad. Sci. USA **103**:10735.
5. Malakhov, M.P. *et al.* (2002) J. Biol. Chem. **277**:9976.
6. Zhang, D. & D.-E. Zhang (2011) J. Interferon Cytokine Res. **31**:119.
7. Jeon, Y.J. *et al.* (2012) J. Clin. Invest. **122**:2622.
8. Harty, R.N. *et al.* (2009) J. Innate. Immun. **1**:397.
9. Owashi, M. *et al.* (2003) Biochem. Biophys. Res. Commun. **309**:533.
10. Bogunovic, D. *et al.* (2012) Science **337**:1684.