

ISG15 [untagged]

Modifying Protein

Alternate Names: G1P2, IFI15, Ubiquitin cross reactive protein

Cat. No. 60-0008-500
Lot. No. 2120

Quantity: 500 µg
Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the ISGylation pathway play a pivotal role in the innate immune response. Three classes of enzymes are involved in the process of ISGylation; the Interferon-stimulated 15 kDa protein (ISG15) activating enzyme UBA7 (E1), the ISG15 conjugating enzyme UBE2L6 (E2) and the ubiquitin like modifier ISG15. ISG15 is a member of the ubiquitin like modifiers and the human gene was first described by Zhao *et al.* (2004). ISG15 functions in various biological pathways from pregnancy to innate immune responses (Ritchie and Zhang. 2004; Zhao *et al.*, 2004). Secretion of ISG15 from monocytes in response to type I Interferons has been shown to cause natural killer (NK) cell proliferation and an increase in non-(major histocompatibility complex) MHC cytotoxicity (Meraro *et al.*, 2002). ISG15 has also been shown to function intracellularly, being covalently conjugated to other proteins by UBA7, UBE2L6 and E3 ligases via a multistep process analogous to ubiquitylation (Loeb and Haas. 1992; Zhao *et al.*, 2005). Ubiquitin-Specific Protease, 43kDa (UBP43) also known as Ubiquitin-Specific Protease, 18 (USP18) has been shown to specifically remove ISG15 from modified proteins (Malakhov *et al.*, 2002).

References:

Loeb KR, Haas AL (1992) The interferon-inducible 15-kDa ubiquitin homolog conjugates to intracellular proteins. *J Biol Chem* **267**, 7806-13.

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 500 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: 17.59 kDa

Purity: >80% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

Protein Sequence:

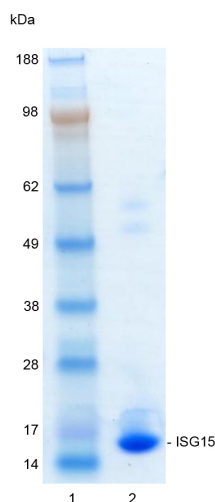
GPLGSMGWDLTVKMLAGNEFQVSLSSSMS
VSELKAQITQKIGVHAFQQRLAVHPS
GVALQDRVPLASQGLGPGSTVLLVVDKCDE
PLNILVRNNKGRSTYEVRLTQTVAHL
KQQVSGLEGVQDDLFWLTFEGKPLEDQL
PLGEYGLKPLSTVFMNLRLRGG

The residues underlined remain after cleavage and removal of the purification tag.
ISG15 (regular text): Start **bold italics** (amino acid residues 1-157)
Accession number: NP_005092

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg ISG15



Protein Identification:

Confirmed by mass spectrometry.

E1 Thioester ISG15 Loading Assay:

The activity of ISG15 was validated by loading ISG15 onto the active cysteine of His-UBA7. Incubation of the His-UBA7 enzyme in the presence of ISG15 and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the ISG15/His-UBA7 thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

Malakhov MP, Malakhova OA, Kim KI, Ritchie KJ, Zhang DE (2002) UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *J Biol Chem* **277**, 9976-81.

Meraro D, Gleit-Kielmanowicz M, Hauser H, Levi BZ (2002) IFN-stimulated gene 15 is synergistically activated through interactions between the myelocyte/lymphocyte-specific transcription factors, PU.1, IFN regulatory factor-8/IFN consensus sequence binding protein, and IFN regulatory factor-4: characterization of a new subtype of IFN-stimulated response element. *J Immunol* **168**, 6224-31.

Ritchie KJ, Zhang DE (2004) ISG15: the immunological kin of ubiquitin. *Semin Cell Dev Biol* **15**, 237-46.

Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, Huibregtse JM, Krug RM (2004) The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. *Proc Natl Acad Sci USA* **101**, 7578-82.

Zhao C, Denison C, Huibregtse JM, Gygi S, Krug RM (2005) Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. *Proc Natl Acad Sci USA* **102**, 10200-5.



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