

# PRODUCT DATA SHEET



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<b>Product name(s):</b>	<b>ISG15 activating enzyme (E1), His<sub>8</sub>-tagged (human, recombinant)</b>				
<b>Product code:</b>	UW9955	<b>Batch number:</b>	Temp	<b>Expiry date:</b>	12 months from receipt

## Product information:

ISG15 is an interferon (IFN)-alpha/beta-induced ubiquitin-like protein that is conjugated to cellular proteins during innate immune responses to bacterial and viral infections, including HIV<sup>1</sup>. Although the biochemical consequences of ISG15 conjugation and the fate of the conjugated proteins are not currently known, it does not appear that ISG15 targets proteins for proteasomal degradation<sup>2,3</sup>.

Conjugation of ISG15 to cellular proteins occurs in a parallel but distinct mechanism to that of ubiquitin<sup>4,5</sup>. The E1 activating enzyme for ISG15, is a single-subunit, ATP dependent enzyme, also known as Ube1L<sup>6</sup> showing a high level of similarity to the E1 activating enzyme for ubiquitin at the protein level. However, this protein does not form a conjugate with ubiquitin, indicating that Ube1L is an E1 enzyme for the ISG15 conjugation system (ISGylation).

UbcH8, a member of the ubiquitin E2-conjugating enzyme family, has been identified as a major E2 enzyme for the ISGylation pathway, interacting with Ube1L to facilitate target protein ISGylation<sup>7,8</sup>. As for ISG15 and Ube1L, UbcH8 expression is induced by IFN<sup>9</sup>.

Recently a number of target proteins conjugated with ISG15 have been identified along with HERC5, an IFN-induced HECT domain-containing E3 protein ligase, shown to be responsible for mediation of cellular protein ISGylation, and itself a target for modification by ISG15<sup>10</sup>.

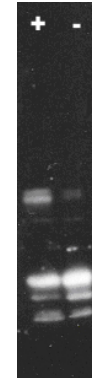
### Data

Accession number: P41226; Length: 1021 amino acid residues; Molecular weight (expressed protein) 113060Da; Tag: C-terminal His<sub>8</sub>; Expression: in *E. coli* BL21 (ΔDE3) Purification: Ni-affinity chromatography – SDS-PAGE, >95% purity.



Coomassie stained gel of ISG15 activating enzyme (E1), His<sub>8</sub>-tagged (human recombinant) (UW9955).

Lane 1: MW markers (top to bottom) 205, 116, 97, 84, 66, 55, 45, 36. Lane 2: UW9955 (2µg).



Thioester activity assay in the presence (+) and absence (-) of ATP. Proteins were detected by Western blotting using ISG15, rabbit polyclonal antibody (PW9575).

UW9955 catalyses formation of a thioester linkage between ISG15 and the E2 conjugating enzyme UbcH8, equivalent to the first step of the ISGylation reaction cascade.

## Storage and use:

Material is supplied at a concentration of 0.21 mg/mL in 50mM Tris-Cl, pH7.5, 50mM NaCl, 1mM DTT. For long-term storage store at -80°C. Avoid multiple freeze/thaw cycles. After initial defrost, aliquot into individual tubes and refreeze at -80°C.

## References:

1. Okumura, A., Lu, G., Pitha-Rowe, I., and Pitha, P. M. Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. *Proc.Natl.Acad.Sci.U.S.A.* **103**, 1440-1445 (2006)
2. Schwartz, D.C. and Hochstrasser, M. A superfamily of protein tags: ubiquitin, SUMO and related modifiers. *Trends Biochem.Sci.* **28**, 321-328 (2003)
3. Malakhov, M.P., Kim, K. I., Malakhova, O. A., Jacobs, B. S., Borden, E. C., and Zhang, D. E. High-throughput immunoblotting. Ubiquitin-like protein ISG15 modifies key regulators of signal transduction. *J.Biol.Chem.* **278**, 16608-16613 (2003)
4. Huang, D.T., Walden, H., Duda, D., and Schulman, B. A. Ubiquitin-like protein activation. *Oncogene.* **23**, 1958-1971 (2004)
5. Loeb, K.R. and Haas, A. L. The interferon-inducible 15-kDa ubiquitin homolog conjugates to intracellular proteins. *J.Biol.Chem.* **267**, 7806-7813 (1992)
6. Yuan, W. and Krug, R. M. Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. *EMBO J.* **20**, 362-371 (2001)
7. Zhao, C., Beaudenon, S. L., Kelley, M. L., Waddell, M. B., Yuan, W., Schulman, B. A., Huibregtse, J. M., and Krug, R. M. The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. *Proc.Natl.Acad.Sci.U.S.A.* **101**, 7578-7582 (2004)
8. Kim, K.I., Giannakopoulos, N. V., Virgin, H. W., and Zhang, D. E. Interferon-inducible ubiquitin E2, Ubc8, is a conjugating enzyme for protein ISGylation. *Mol.Cell Biol.* **24**, 9592-9600 (2004)
9. Nyman, T.A., Matikainen, S., Sareneva, T., Julkunen, I., and Kalkkinen, N. Proteome analysis reveals ubiquitin-conjugating enzymes to be a new family of interferon-alpha-regulated genes. *Eur.J.Biochem.* **267**, 4011-4019 (2000)
10. Wong, J.J., Pung, Y. F., Sze, N. S., and Chin, K. C. HERC5 is an IFN-induced HECT-type E3 protein ligase that mediates type I IFN-induced ISGylation of protein targets. *Proc.Natl.Acad.Sci.U.S.A.* **103**, 10735-10740 (2006)