

IpaH9.8 [GST-tagged]

E3 Ligase

Alternate Name: Invasion plasmid antigen IpaH9.8

Cat. No. 63-0018-025

Lot. No. 30024

Quantity: 25 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 ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). IpaH9.8 is a bacterial E3 ligase and cloning of the gene from *Shigella flexneri* was first described by Demers *et al.* (1998). The IpaH family of proteins contain highly conserved leucine-rich repeats (LRR) in the amino-terminal region while the conserved carboxy-terminal regions possess E3 ligase activity (Rohde *et al.*, 2007). The crystal structure of the IpaH protein has recently been described and based on this the IpaH family has been identified as a new class of E3 ubiquitin ligases found in pathogenic and symbiotic bacteria (Singer *et al.*, 2008; Zhu, *et al.*, 2008). IpaH9.8 has been shown to regulate the pheromone response by ubiquitylation and degradation of the MAPK kinase Ste7 (Rohde *et al.*, 2007). IpaH9.8 has also been shown to inhibit NF-κB-dependent gene expression and the activity of U2AF35, an alternative splicing factor involved in host inflammatory responses (Haraga and Miller 2003).

Continued on page 2

Physical Characteristics

Species: *Shigella flexneri*

Source: *E. coli* expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~88.8 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

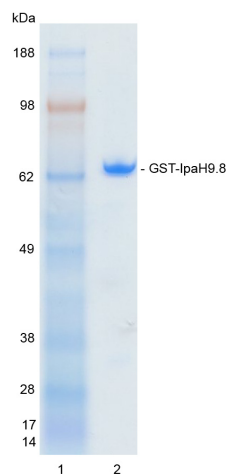
Quality Assurance

Protein Identification:

Confirmed by mass spectrometry.

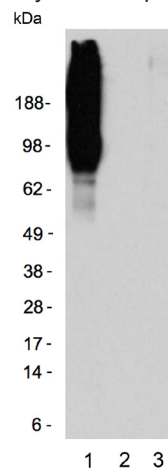
Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-IpaH9.8



E3 ligase assay:

The ubiquitin conjugating activity of GST-IpaH9.8 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D2 (UbcH5b) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-IpaH9.8 for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D2 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-IpaH9.8 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and GST-IpaH9.8.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

References:

Demers B, Sansonetti PJ, Parsot C (1998) Induction of type III secretion in *Shigella flexneri* is associated with differential control of transcription of genes encoding secreted proteins. *EMBO J* 17, 2894-903.

Haraga A, Miller SI (2003) A *Salmonella enterica* serovar typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. *Infect Immun* 71, 4052-8.

Rohde JR, Breittkreutz A, Chenal A, Sansonetti PJ, Parsot C (2007) Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. *Cell Host Microbe* 1, 77-83.

Singer AU, Rohde JR, et al. (2008) Structure of the *Shigella* T3SS effector IpaH defines a new class of E3 ubiquitin ligases. *Nat Struct Mol Biol* 15, 1293-301.

Zhu Y, Li H, Hu L, Wang J, Zhou Y, Pang Z, Liu L, Shao F (2008) Structure of a *Shigella* effector reveals a new class of ubiquitin ligases. *Nat Struct Mol Biol* 15, 1302-8.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKY
EEHLIERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTSMAIIRYIADKHN
MLGGCPKERAEISMLEGAVLDIRYGV
RIAYSKDFETLKVDFLSKLPEMLKMF
DRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPO
IDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFGQPLGSMPLPINNNFSLPQNS
FYNTISGTYADYFSAWDKWEKQALPG
EERDEAVSRLKECLINNSDELRLDRLNLS
LPDNLPAQITLLNVSYNQLTNLPELPVTLK
KLYSASNKLSLELPVLPALLESLOVQH
NELENLPALPDSLTLTMNISYNEIVS
LPSLPQALKNLRATRNFTELPAFSEGN
NPVVREYFFDRNQISHIPESILNLRNECSI
HISDNPLSSHALQALQRLTSSPDYHGPR
YFSMSDGGQNTLHRPLADAVTAWFPENKQS
DVSQIWHAFEEEEHANTFSAFLDRLSDT
VSARNTSGFREQVAAWLEKLSASAELRQOS
FAVAADATESCEDRVALTWNNLRKTL
VHQASEGLFDNDTGALLSLGREMFREILE
DIARDKVRTLHFVDEIEVYLAFQTM
LAEKQLSTAVKEMRFYGVSGVTANDLR
TAEAMVRSRENEFTDWFSLWGPWHAV
LKRTEADRWALAEQKYEMLENEYPQR
VADRLKASGLSGDADAEREAGAQM
RETEQQIYRQLTDEVLALRLPENGSQLHHS

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVL**FQ**▼GP)
IpaH9.8 (regular text): Start **bold italics** (amino acid residues 1-545)
Accession number: AAP79029.1



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