

NLEL [GST-tagged]

E3 Ligase

Alternate Names: Non-LEE-encoded Ligase

Cat. No. 63-0038-025

Lot. No. 30213

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). Non-LEE-encoded ligase (NleL) is a member of the E3 protein ligase family and cloning of the gene from *Escherichia coli* was first described by Kulasekara *et al.* (2009). Many pathogenic bacteria can deliver virulence factors into host cells that function as E3 ligases and NleL is a bacterial ubiquitin E3 ligase involved in pedestal formation (Lin *et al.*, 2012; Piscatelli *et al.*, 2011). NleL has been shown to contain a cysteine residue near the C terminus of the protein that forms a transient thioester bond with Ubiquitin (Piscatelli *et al.*, 2011). Similar to eukaryotic HECT E3s ligases, NleL functions with a subgroup of E2 enzymes that contain a conserved phenylalanine residue (Lin *et al.*, 2010). NleL also possesses the conformational flexibility characteristic of HECT E3 ligases, however, the molecular surface of NleL bears no similarity to that of HECT E3 ligases (Daio *et al.*, 2008; Lin *et al.*, 2010).

References:

Diao J, Zhang Y, Huijbregtse JM, Zhou D, Chen J (2008) Crystal structure of SopA, a Salmonella effector protein mimicking a eukaryotic ubiquitin ligase. *Nat Struct Mol Biol* 15, 65–70.

Kulasekara BR, Jacobs M, Zhou Y, Wu Z, Sims E, *et al.* (2009) Analysis of the genome of the *Escherichia coli* O157:H7 2006 spinach-associated outbreak isolate indicates candidate genes that may enhance virulence. *Infect Immun* 77, 3713–3721.

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Physical Characteristics

Species: *Escherichia coli*

Source: *E. coli*

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: ~107 kDa

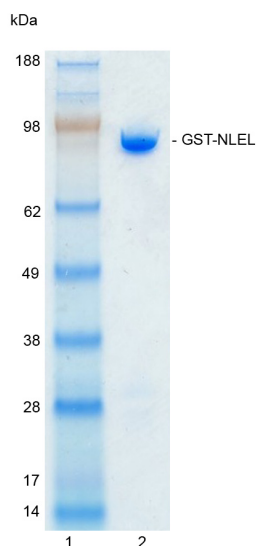
Purity: >95% by InstantBlue™ SDS-PAGE

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

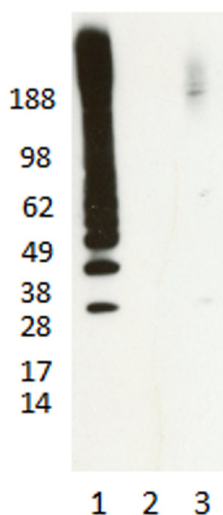
Protein Sequence: Please see page 2

Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-NLEL 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-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-NLEL for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-NLEL (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-NLEL.



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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

Lin DY, Diao J, Zhou D, Chen J (2010) Biochemical and structural studies of a HECT-like ubiquitin ligase from *Escherichia coli* O157:H7. *J Biol Chem* **286**, 441-449.

Lin DY, Diao J, Chen J (2012) Crystal structures of two bacterial HECT-like E3 ligases in complex with a human E2 reveal atomic details of pathogen-host interactions. *PNAS* **109**, 1925-30.

Piscatelli H, Kotkar SA, McBee ME, Muthupalani S, Schauer DB, Mandrell RE, Leong JM, Zhou D (2011) The EHEC type III effector NleL is an E3 ubiquitin ligase that modulates pedestal formation. *PLoS One* **6**, e19331.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSR IAY
SKDFETLKVDFLSKLP EMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYM
DPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDEN
LYFQGGSNGETLSISEPITTLPDLLPG
SLKELVLNGCTELKSINCLPPNLSLSM
VGCSSLEVINC SIPENVINLSLCHCSS
LKHIEGSFPEALRNSVYLNCGNSL NESQC
QFLAYDVSQGRACL SKAELTADLIWLSAN
RTGEESA EELNYS GCDLSGLSLVGLNLS
VNFSGAVLDDTLRMSDLSQAVLENC SFKN
SILNECNFCYANLSNCIIRALFENS NFSN
SNLKNASF KGSYIQYPPILNEADLTGAI I
IPGMVLSGAILGDVKELFSEKSNTIN
LGGCYIDLSDIQENILSVLDNYTKSNK
SILLTMNTSDDKYNHDKVRAAEELIK
KISLDELAAFRPYVKMSLADSF S I H
PYLNNANIQQWLEPICDDFFDTIMSWF
NNSIMMYMENGSL LQAGMYFERHPGAM
VSYNSSFIQIVMNGSR RDGMQERFRELY
EVYLNKNEKVPVTQQSDFGLCDGSGKPDWD
DSDLAYNWVLLSSQDDGMAMMCSLSHMVD
MLSPNTSTNWM SFFLYKDG EVQNTFGYSL
SNLFS ESFP IFSIPYHKAFSQNFVSGILD
ILISDNELKERFIEALNSNKSDYKMIAD
DQQRKLACVWNPFLDGWELNAQHVD MIMGSH
VLKDMPLRKQAEILFCLGGVFCKYSSSDMF
GTEYD SPEILRRYANGLIEQAYKTDPOVFGS
VYYYNDILDR LQGRNNVFTCTAVLTDMLTE
HAKESFPEIFSLYYPVAWR

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (**ENLYFQ▼G**)
NLEL (regular text): Start **bold italics** (amino acid residues 59-782)
Accession number: NP_309587.1



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