

NEMO (D311N) [GST-tagged]

Ubiquitin Binding Protein

Alternate Name: IKBKKG, NFkB essential modulator

Cat. No. 66-1013-050

Lot. No. 30101

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Ubiquitin signals are decoded in cells by at least 200 ubiquitin binding proteins, which interact with different types of polyubiquitin chains and ubiquitin-like modifiers. These interactions induce conformational changes that allow these proteins to transmit the ubiquitin signal to effector proteins (Dikic *et al.*, 2009). NEMO (NFkB Essential Modifier) is the prototypic member of a family of proteins that interact with Lys63-linked and linear polyubiquitin chains (Nanda *et al.*, 2011). (Nanda *et al.*, 2011). NEMO functions as a high affinity receptor for linear ubiquitin chains and a low affinity receptor for long lysine-linked ubiquitin chains. It is thought that this phenomenon could explain quantitatively distinct NF-κB activation patterns in response to numerous cell stimuli (Kensche *et al.*, 2012). It is an integral component of the canonical IκB kinase (IKK) complex and is essential for the activation of IKKα and IKKβ, the protein kinase components of the complex. Mutations that abrogate binding of polyubiquitin chains to NEMO do not activate the IKK complex (Ea *et al.*, 2006; Wu *et al.*, 2006) and cause a severe immunodeficiency disease and greatly increased susceptibility to infection by bacteria of the tuberculosis family (Doffinger *et al.*, 2001). NEMO also interacts with TANK, a component of the IKK-related kinases TBK1 and IKKε (Chariot *et al.*, 2002). The NEMO-TANK interaction is essential for effective cross-talk between the canonical IKK complex and the IKK-related kinases which, if disrupted by the loss of TANK, leads to the hyperactivation of the innate immune system and to

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

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

Protein Sequence: Please see page 2

Molecular Weight: ~74.9 kDa

Purity: >85% by InstantBlue™ SDS-PAGE

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

Quality Assurance

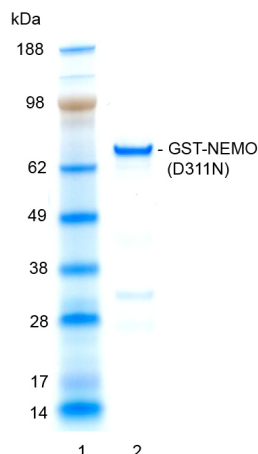
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg GST-NEMO (D311N)

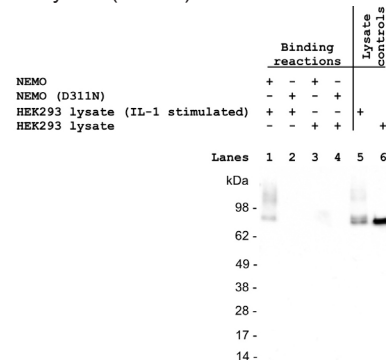


Protein Identification:

Confirmed by mass spectrometry.

Ubiquitin Binding Domain Activity:

The lack of GST-NEMO (D311N) ubiquitin chain binding domain activity was determined through its inability to capture polyubiquitylated IRAK1 from a lysate preparation derived from IL-1 stimulated HEK293 cells. GST-NEMO (D311N) was pre-incubated with Glutathione Sepharose 4B for 20 minutes at 4°C followed by an incubation for 2 hours at 4°C with 2mg IL-1 stimulated HEK293 cell lysate. The binding reaction was then centrifuged and the pellet analysed by SDS-PAGE/Western blotting (Lane 2). This sample was compared alongside similarly derived pull-downs from control reactions containing GST-NEMO wild-type versus mutant (D311N) incubated in the presence of lysates derived from either IL-1 stimulated or non-stimulated HEK293 cells (Lanes 1-4). Ubiquitylated IRAK1 was identified by Western Blotting using an anti-IRAK1 antibody and such species were observed only in the pellet sample derived from a binding reaction containing wild-type GST-NEMO and IL-1 stimulated HEK293 cell lysate (Lane 1).



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2012. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

NEMO (D311N) [GST-tagged]

Ubiquitin Binding Protein

Alternate Name: IKBK, NFkB essential modulator

Cat. No. 66-1013-050

Lot. No. 30101

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

autoimmune disease (Clark *et al.*, 2011; Kawagoe *et al.*, 2009). NEMO is a powerful reagent for capturing the Lys63-linked and linear polyubiquitin chains and their binding partners present in cell extracts. It is recommended that the NEMO [D311N] mutant, which is unable to bind polyubiquitin chains, is used as a control in such experiments (Windheim *et al.*, 2008).

References:

Chariot A, Leonardi A, Muller J, Bonif M, Brown K and Siebenlist U (2002) Association of the adaptor TANK with the I kappa B kinase (IKK) regulator NEMO connects IKK complexes with IKK epsilon and TBK1 kinases. *J Biol Chem* 277, 37029-37036.

Clark K, Takeuchi O, Akira S and Cohen P (2011) The TRAF-associated protein TANK facilitates cross-talk within the I kappa B kinase family during Toll-like receptor signaling. *Proc Natl Acad Sci U S A* 108, 17093-17098.

Dikic I, Wakatsuki S and Walters KJ (2009) Ubiquitin-binding domains - from structures to functions. *Nat Rev Mol Cell Biol* 10, 659-671.

Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, *et al.* (2001) X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nature Genetics* 27, 277-285.

Ea CK, Deng L, Xia ZP, Pineda G and Chen ZJ (2006) Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Molecular Cell* 22, 245-257.

Kawagoe T, Takeuchi O, Takabatake Y, Kato H, Isaka Y, Tsujimura T, *et al.* (2009) TANK is a negative regulator of Toll-like receptor signaling and is critical for the prevention of autoimmune nephritis. *Nature Immunology* 10, 965-972.

Kensche T, Tokunaga F, Ikeda F, Goto E, Iwai K and Dikic I (2012) Analysis of NF-kappaB essential modulator (NEMO) binding to linear and lysine-linked ubiquitin chains and its role in the activation of NF-kappaB. *J Biol Chem* 287, 23626-34.

Nanda SK, Venigalla RK, Ordureau A, Patterson-Kane JC, Powell DW, Toth R, *et al.* (2011) Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. *J Exp Med* 208, 1215-1228.

Windheim M, Stafford M, Pegg M and Cohen P (2008) Interleukin-1 (IL-1) induces the Lys63-linked polyubiquitination of IL-1 receptor-associated kinase 1 to facilitate NEMO binding and the activation of I kappa B alpha kinase. *Mol Cell Biol* 28, 1783-1791.

Wu CJ, Conze DB, Li T, Srinivasula SM and Ashwell JD (2006) Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation [corrected]. *Nature Cell Biology*, 8, 398-406.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCCKERAIEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLEVLFGPLGSNRHLWKSQ
CEMVQPSGGPAADQDVLGEE SPLGKPAML
HLPSEQGAPETLQRCLEENQELRDAIRQSNQ
ILRERCEELLHFQASQREEKEFLMCKFQEAR
KLVERLGLKLDLKRQKEQALREVEHLKRC
QQQMAEDKASVKAQVTSLLGELQESQSR
LEAATKECQALEGRARAASEQARQLE SERE
ALQQQHSVQVDQLRMQGSVEAALRMERQAA
SEEKRKLAQLQVAYHQLFQYEDNHIKSSVVG
SEKRGMQLEDLQQLQQAEEALVAKQAEVI
DKLKEEAEQHKIVMETVPLKAQADIYKAN
FQAERQAREKLEAKKELLQEQLEQLQREY
SKLKASCQESARIEDMRKRHVEVSQAPLPPA
PAYLSSPLALPSQRRSPPEEPPDFCCPKCQY
QAPDMDTLQIHVMCEIE

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVLFG▼GP)

NEMO (D311N) (regular text): Start **bold italics**

(amino acid residues 2-418)

Accession number: AAD38081



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2012. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0