NEMO [GST-tagged]

Ubiquitin Binding Protein

Alternate Name: IKBKG, NFkB essential modulator

Cat. No. **66-1002-050**
Lot. No. **30058**

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 (NFκB Essential Modifier) is the protypic member of a family of proteins that interact with Lys63-linked and linear polyubiquitin chains (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). NEMO 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 autoimmune disease (Clark *et al.*, 2011;

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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

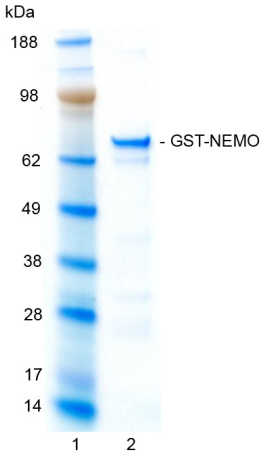
Molecular Weight: ~74.9 kDa

Purity: >85% by InstantBlue™ SDS-PAGE

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

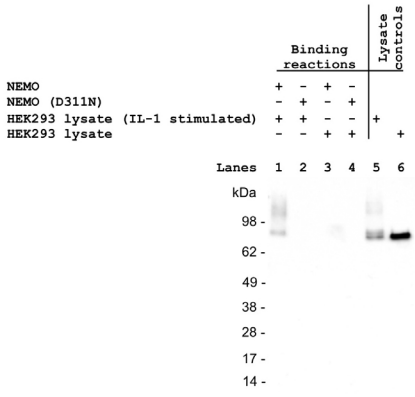
Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.

Ubiquitin Binding Domain Activity:
The ubiquitin chain binding domain activity of GST-NEMO was validated through its ability to capture poly-ubiquitylated IRAK1 from a lysate preparation derived from IL-1 stimulated HEK293 cells. GST-NEMO was pre-incubated with Glutathione Sepharose 4B for 20 minutes at 4˚C followed by 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 1). 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 2-4). 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).



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Lot-specific COA version tracker: v1.0.0

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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 IkappaB 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, Peggie 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 IkappaBalpha 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:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLEVLFQGPLGS***N***RHLWKSQL
CEMVQPSGGPAADQDVLGEESPLGKPAML
HLPSEQGAPETLQRCLEENQELRDAIRQSNQ
ILRERCEELLHFQASQREEKEFLMCKFQEAR
KLVERLGLEKLDLKRQKEQALREVEHLKRC
QQQMAEDKASVKAQVTSLLGELQESQSR
LEAATKECQALEGRARAASEQARQLESERE
ALQQQHSVQVDQLRMQGQSVEAALRMERQAA
SEEKRKLAQLQVAYHQLFQEYDNHIKSSVVG
SERKRGMQLEDLKQQLQQAEEALVAKQEVI
DKLKEEAEQHKIVMETVPVLKAQADIYKAD
FQAERQAREKLAEKKELLQEQLEQLQREY
SKLKASCQESARIEDMRKRHVEVSQAPLPPA
PAYLSSPLALPSQRRSPPEEPPDFCCPKCQY
QAPDMDTLQIHVMECIE

Tag **(bold text):** N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
NEMO (regular text): Start ***bold italics*** (amino acid residues 2-418)
Accession number: AAD38081

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