NEMO (human; full length), pAb

Alternate Names: NF-kappa-B essential modulator, FIP-3, IkB kinase-associated protein 1, Inhibitor of nuclear factor kappa-B kinase subunit gamma.

Cat. No.	**68-0014-100**	Quantity:	100 µg
Lot. No.	**30251**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

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 prototypic 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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human NEMO (residues 2-419) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects NEMO at ~48 kDa

Reactivity: human

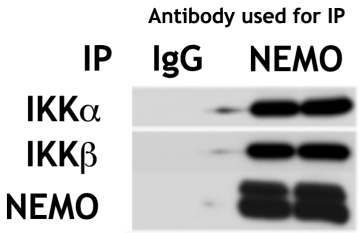
Species cross reactivity: mouse

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Not tested

Immunoprecipitation:
Use 5 µg/mg of cell extract



Immunoprecipitation Assay:
NEMO was immunoprecipitated from RAW264.7 total cell extracts (1 mg) using 5 µg anti-NEMO antibody (Cat# 68-0014-100). NEMO was not immunoprecipitated using a control antibody (IgG). NEMO was subsequently detected by a commercially sourced anti-NEMO antibody.

N.B: As NEMO is a component of the canonical IKK complex, other components of the complex (the catalytic subunits IKKα and IKKβ) are also immunoprecipitated with the anti-NEMO antibody.

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

Lot-specific COA version tracker: v1.0.0

NEMO (human; full length), pAb

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Background

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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; Kawagoe *et al.*, 2009).

Antibody Production:
Anti-NEMO (human) polyclonal antibody was raised in sheep against NEMO (residues 2-419 of human NEMO). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-NEMO pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-NEMO (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General 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.

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. *Nature Cell Biology* **8**, 398-406

Application References:

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.

Emmerich CH, Ordureau A, Strickson S, Arthur JSC, Pedriolo PGA, Komander D and Cohen P (2013) Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *PNAS* **110**, 15247-52.

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