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Datasheet for 200-401-GM7

NEMO/IKK-gamma Antibody

Overview

Description:	Anti-NEMO (RABBIT) Antibody - 200-401-GM7
Item No.:	200-401-GM7
Size:	100 µg
Applications:	WB, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti-NEMO antibody was designed, produced, and validated as part of the Joy Cappel Young Investigator Award (JCYIA). Anti-NEMO antibody detects recombinant and endogenous NEMO. NEMO, the regulatory subunit of the IKK core complex, phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Its binding to scaffolding polyubiquitin seems to play a role in IKK activation by multiple signaling receptor pathways. Nemo is also considered to be a mediator for TAX activation of NF-kappa-B and may be implicated in NF-kappa-B-mediated protection from cytokine toxicity. NEMO is essential for viral activation of IRF3 and involved in TLR3- and IFIH1-mediated antiviral innate response. The innate antiviral response from NEMO requires 'Lys-27'-linked polyubiquitination. Anti-NEMO is ideal for researchers interested in Immunology and Cancer research.
Synonyms:	rabbit anti-NEMO antibody, IKBKG Antibody, NF-kappa-B essential modulator, FIP3, NEMO, regulatory subunit of the IKK core complex, I-kappa-B kinase subunit gamma, IKK-gamma, IKKG, IkB kinase-associated protein 1, IKKAP1, rabbit anti-IKK gamma
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	IKBKG
Reactivity:	Human

Immunogen Type:	Recombinant Protein
Immunogen:	Anti-NEMO was affinity purified from whole rabbit serum prepared by repeated immunizations with a recombinant protein of human NEMO.
Purity/Specificity:	Anti-NEMO is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. This antibody detects human NEMO. Cross reactivity with NEMO from other sources is unknown.
Relevant Links:	• UniProtKB - Q9Y6K9 • GeneID - 8517 • NCBI - NP_003630.1

Application Details

Tested Applications:	WB
Suggested Applications:	IP (Based on references)
Application Note:	Anti-NEMO antibody has been tested by western blot and is suitable for immunoprecipitation and ELISA. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 48kDa in size corresponding to endogenous NEMO protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:50,000-1:400,000
IP:	5µg
WB:	1:500-1:1000

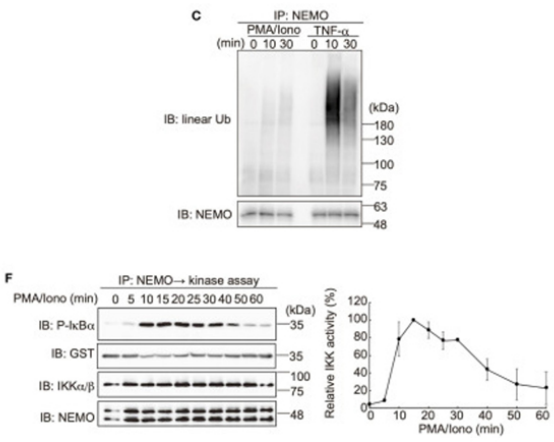
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.1 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

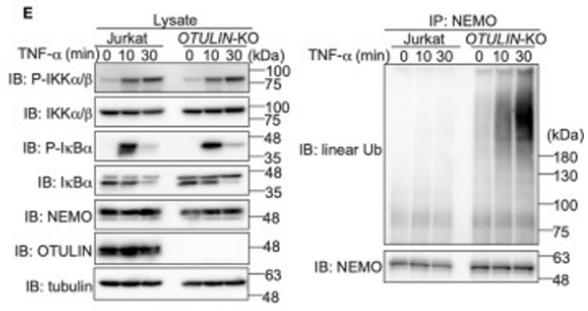
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

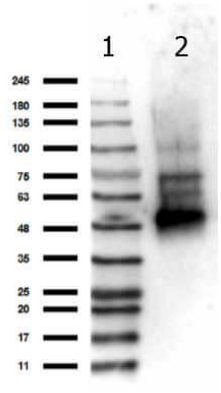
Images



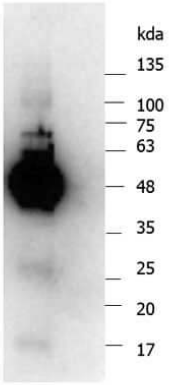
Western Blot
The CBM complex is linearly ubiquitinated upon TCR stimulation. (C) Suppressed linear ubiquitination of NEMO in the TCR-mediated NF-κB activation pathway. Jurkat cells were stimulated with 20 ng/ml PMA and 150 ng/ml ionomycin or 1 µg/ml TNF-α for the indicated time periods, and cell lysates were immunoprecipitated with an anti-NEMO antibody and then immunoblotted with the depicted antibodies. (F) Transient activation of canonical IKK. Jurkat cells were stimulated with 20 ng/ml PMA and 150 ng/ml ionomycin for the indicated time periods. After immunoprecipitation with an anti-NEMO antibody, an in vitro canonical IKK assay was performed using GST-IκBα1–54 as the substrate. Samples were immunoblotted with the indicated antibodies, and taking the maximum intensities of P-IκBα as 100%, the relative intensities are indicated. Means ±SD (n = 3). Fig 4. PMID: 33329596



Western Blot
OTULIN predominantly downregulates TCR-mediated NF-κB activation in Jurkat cells. (E) Enhanced linear ubiquitination of NEMO in OTULIN-KO cells upon TNFα treatment. Jurkat and OTULIN-KO cells were stimulated with 1 µg/ml TNF-α for the indicated time periods and analyzed as in Figure 4C . Fig 5. PMID: 33329596



Western Blot
Western Blot of Rabbit anti-NEMO antibody. Lane 1: Opal Pre-stained ladder (p/n MB-210-0500). Lane 2: Recombinant NEMO protein. Load: 175 ng per lane. Primary antibody: NEMO antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:70,000 for 30 min at RT. Blocking Buffer: MB-070 for 30 min at RT. Predicted MW: ~55kDa. Observed MW: ~50kDa for NEMO.



Western Blot
Western Blot of Rabbit anti-NEMO antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: Recombinant NEMO protein. Load: 50 ng per lane. Primary antibody: NEMO antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 30 min at RT. Blocking Buffer: MB-070 for 30 min at RT. Predicted MW: ~55kDa. Observed MW: ~48kDa for NEMO.

References

- Oikawa D et al. Cellular and Mathematical Analyses of LUBAC Involvement in T Cell Receptor-Mediated NF-κB Activation Pathway. *Front Immunol.* (2020)

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