

**Datasheet for 200-401-GM7S****NEMO/IKK-gamma Antibody****Overview**

|                      |                                            |
|----------------------|--------------------------------------------|
| <b>Description:</b>  | Anti-NEMO (RABBIT) Antibody - 200-401-GM7S |
| <b>Item No.:</b>     | 200-401-GM7S                               |
| <b>Size:</b>         | 25 µL                                      |
| <b>Applications:</b> | WB, IP                                     |
| <b>Reactivity:</b>   | Human                                      |
| <b>Host Species:</b> | Rabbit                                     |

**Product Details**

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background:</b>   | 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. |
| <b>Synonyms:</b>     | 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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Host Species:</b> | Rabbit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Clonality:</b>    | Polyclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Format:</b>       | IgG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

**Target Details**

|                    |       |
|--------------------|-------|
| <b>Gene Name:</b>  | IKBKG |
| <b>Reactivity:</b> | Human |

|                            |                                                                                                                                                                                                                                                                                                                                                |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Immunogen Type:</b>     | Recombinant Protein                                                                                                                                                                                                                                                                                                                            |
| <b>Immunogen:</b>          | Anti-NEMO was affinity purified from whole rabbit serum prepared by repeated immunizations with a recombinant protein of human NEMO.                                                                                                                                                                                                           |
| <b>Purity/Specificity:</b> | 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. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q9Y6K9</a></li><li>• <a href="#">GeneID - 8517</a></li><li>• <a href="#">NCBI - NP_003630.1</a></li></ul>                                                                                                                                                                      |

## Application Details

|                                |                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b>    | WB                                                                                                                                                                                                                                                                                                                              |
| <b>Suggested Applications:</b> | IP (Based on references)                                                                                                                                                                                                                                                                                                        |
| <b>Application Note:</b>       | 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. |
| <b>Assay Dilutions:</b>        | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                 |
| <b>ELISA:</b>                  | 1:50,000-1:400,000                                                                                                                                                                                                                                                                                                              |
| <b>IP:</b>                     | 5µg                                                                                                                                                                                                                                                                                                                             |
| <b>WB:</b>                     | 1.0 ug/ml                                                                                                                                                                                                                                                                                                                       |

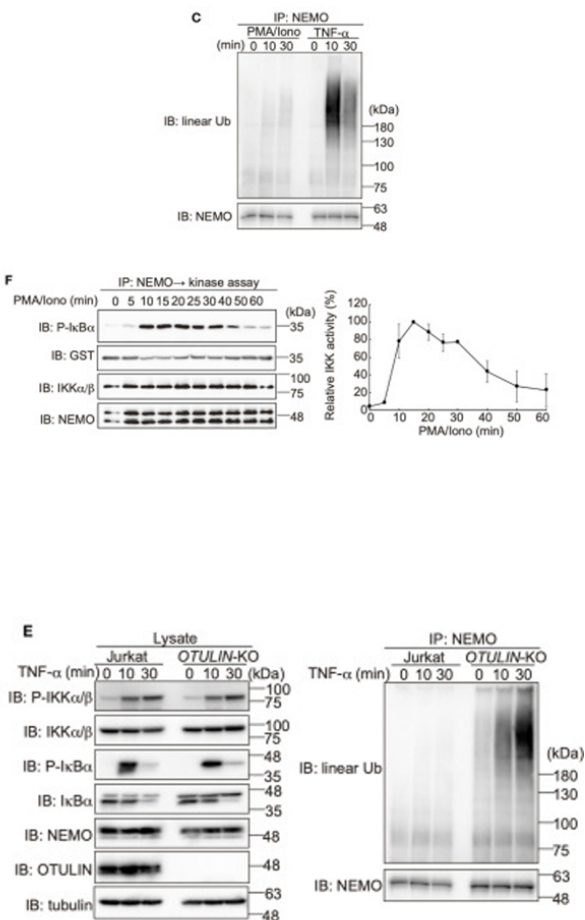
## Formulation

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

## Shipping & Handling

|                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Shipping Condition: | Dry Ice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Storage Condition:  | Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing. |
| Expiration:         | Expiration date is one (1) year from date of receipt.                                                                                                                                                                                                                                                                                                                                                                                                                                              |

Images

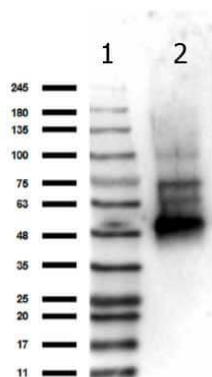


Western Blot

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

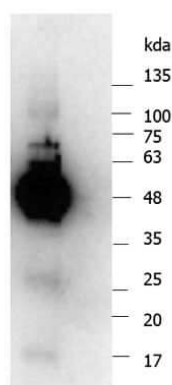
Western Blot

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- $\kappa$ B Activation Pathway. *Front Immunol.* (2020)

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.