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Anti-NMI Antibody Picoband™ (monoclonal, 2F3)

Catalog # ABO14854

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q13287
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-NMI Antibody Picoband™ (monoclonal, 2F3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	9111
Other Names	N-myc-interactor, Nmi, N-myc and STAT interactor, NMI (HGNC:7854)
Calculated MW	35057
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 5 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cytoplasm.
Tissue Specificity	Expressed in all adult and fetal tissues except brain and skin. More abundant in fetal tissues especially liver.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 2F3
Immunogen	E.coli-derived human NMI recombinant protein (Position: E2-E307). Human NMI shares 64% amino acid (aa) sequence identity with mouse NMI.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six

months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	NMI (HGNC:7854)
Function	Acts as a signaling pathway regulator involved in innate immune system response (PubMed:26342464, PubMed:29038465, PubMed:29350881, PubMed:9989503). In response to interleukin 2/IL2 and interferon IFN-gamma/IFNG, interacts with signal transducer and activator of transcription/STAT which activate the transcription of downstream genes involved in a multitude of signals for development and homeostasis (PubMed:29377960, PubMed:9989503). Enhances the recruitment of CBP/p300 coactivators to STAT1 and STAT5, resulting in increased STAT1- and STAT5-dependent transcription (PubMed:9989503). In response to interferon IFN-alpha, associates in a complex with signaling pathway regulator IFI35 to regulate immune response; the complex formation prevents proteasome-mediated degradation of IFI35 (PubMed:10779520, PubMed:10950963). In complex with IFI35, inhibits virus-triggered type I IFN-beta production when ubiquitinated by ubiquitin-protein ligase TRIM21 (PubMed:26342464). In complex with IFI35, negatively regulates nuclear factor NF-kappa-B signaling by inhibiting the nuclear translocation, activation and transcription of NF-kappa-B subunit p65/RELA, resulting in the inhibition of endothelial cell proliferation, migration and re-endothelialization of injured arteries (PubMed:29350881). Negatively regulates virus-triggered type I interferon/IFN production by inducing proteosome-dependent degradation of IRF7, a transcriptional regulator of type I IFN, thereby interfering with cellular antiviral responses (By similarity). Beside its role as an intracellular signaling pathway regulator, also functions extracellularly as damage-associated molecular patterns (DAMPs) to promote inflammation, when actively released by macrophage to the extracellular space during cell injury or pathogen invasion (PubMed:29038465). Macrophage-secreted NMI activates NF-kappa-B signaling in adjacent macrophages through Toll-like receptor 4/TLR4 binding and activation, thereby inducing NF-kappa-B translocation from the cytoplasm into the nucleus which promotes the release of pro-inflammatory cytokines (PubMed:29038465).
Cellular Location	Cytoplasm. Nucleus. Secreted Note=Cytoplasmic NMI localizes in punctate granular structures (PubMed:10950963, PubMed:9781816). Nuclear localization increased following IFN-alpha treatment (PubMed:10950963, PubMed:9781816) Extracellular following secretion by macrophage (PubMed:29038465)
Tissue Location	Expressed in adult spleen, liver, and kidney (PubMed:9781816). Expressed in fetal thymus, liver, placenta, spleen, lung, and kidney but not brain (PubMed:9781816). Expressed in macrophages (PubMed:29038465).

Background

NMYC interactor (NMI) encodes a protein that interacts with NMYC and CMYC (two members of the oncogene Myc family), and other transcription factors containing a Zip, HLH, or HLH-Zip motif. The NMI protein also interacts with all STATs except STAT2 and augments STAT-mediated transcription in response to cytokines IL2 and IFN-gamma. The NMI mRNA has low expression levels in all human fetal and adult tissues tested except brain and has high expression in cancer cell line-myeloid leukemias.

Images

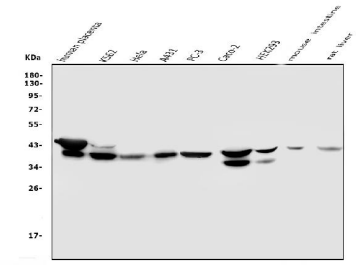


Figure 1. Western blot analysis of NMI using anti-NMI antibody (M02768).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human placenta tissue lysates,
Lane 2: K562 whole cell lysates,
Lane 3: Hela whole cell lysates,
Lane 4: A431 whole cell lysates,
Lane 5: PC-3 whole cell lysates,
Lane 6: Caco-2 whole cell lysates,
Lane 7: HEK293 whole cell lysates,
Lane 8: mouse intestine tissue lysates,
Lane 9: rat liver tissue lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-NMI antigen affinity purified monoclonal antibody (Catalog # M02768) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for NMI at approximately 40KD. The expected band size for NMI is at 35KD.

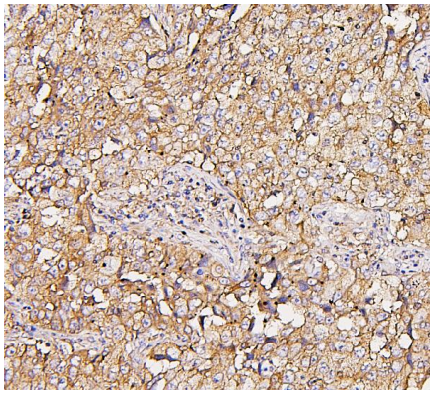


Figure 2. IHC analysis of NMI using anti-NMI antibody (M02768).
NMI was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-NMI Antibody (M02768) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

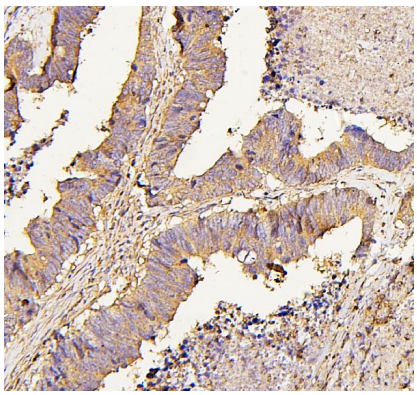
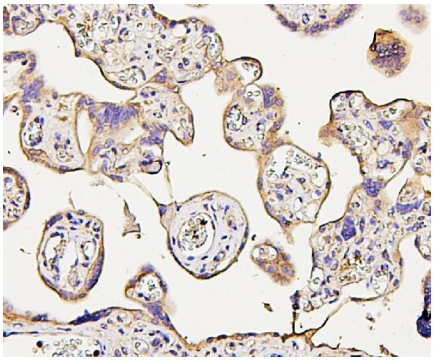


Figure 3. IHC analysis of NMI using anti-NMI antibody (M02768).
NMI was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-NMI Antibody (M02768) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 4. IHC analysis of NMI using anti-NMI antibody



(M02768).
NMI was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-NMI Antibody (M02768) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

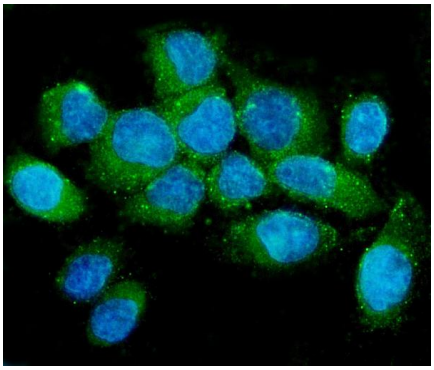


Figure 5. IF analysis of NMI using anti-NMI antibody (M02768).
NMI was detected in immunocytochemical section of SiHa cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-NMI Antibody (M02768) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

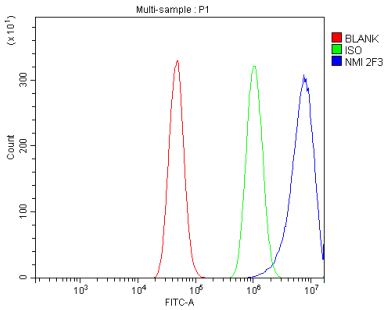


Figure 6. Flow Cytometry analysis of A431 cells using anti-NMI antibody (M02768).
Overlay histogram showing A431 cells stained with M02768 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-NMI Antibody (M02768, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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