10320 Camino Santa Fe, Suite G
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Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7)

Catalog # ABO16563

Specification

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	Q9NV92
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Additional Information

Gene ID 54602

Other Names
NEDD4 family-interacting protein 2, NEDD4 WW domain-binding protein 5A, Putative MAPK-activating protein PM04/PM05/PM06/PM07, Putative NF-kappa-B-activating protein 413, NDFIP2, KIAA1165, N4WBP5A

Calculated MW
39 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
E.coli-derived human NDFIP2 recombinant protein (Position: M16-L336).

Purification
Immunogen affinity purified.

Storage	**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**

freezing and thawing.

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Protein Information

Name NDFIP2

Synonyms KIAA1165, N4WBP5A

Function
Activates HECT domain-containing E3 ubiquitin-protein ligases, including ITCH, NEDD4, NEDD4L, SMURF2, WWP1 and WWP2, and consequently modulates the stability of their targets. As a result, may control many cellular processes. Recruits ITCH, NEDD4 and SMURF2 to endosomal membranes. Negatively regulates KCNH2 potassium channel activity by decreasing its cell-surface expression and interfering with channel maturation through recruitment of NEDD4L to the Golgi apparatus and multivesicular body where it mediates KCNH2 degradation (PubMed:26363003). May modulate EGFR signaling. Together with NDFIP1, limits the cytokine signaling and expansion of effector Th2 T-cells by promoting degradation of JAK1, probably by ITCH- and NEDD4L-mediated ubiquitination (By similarity).

Cellular Location
Endosome membrane; Multi-pass membrane protein. Golgi apparatus membrane. Endosome, multivesicular body membrane

Tissue Location
Expressed in brain, lung, heart, skeletal muscle, kidney, liver and placenta.

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Images

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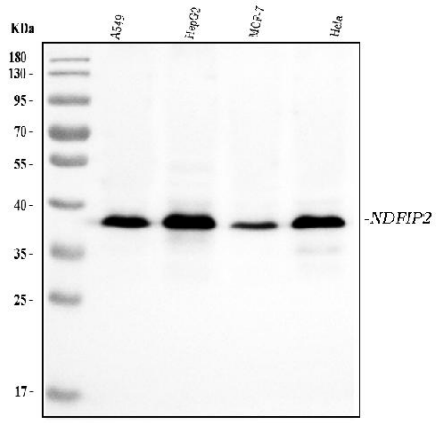

Figure 1. Western blot analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human A549 whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human MCF-7 whole cell lysates,
Lane 4: human Hela whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-NDFIP2 antigen affinity purified monoclonal antibody (Catalog # M08384) 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 1:10000 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 NDFIP2 at approximately 39 kDa. The expected band size for NDFIP2 is at 36 kDa.

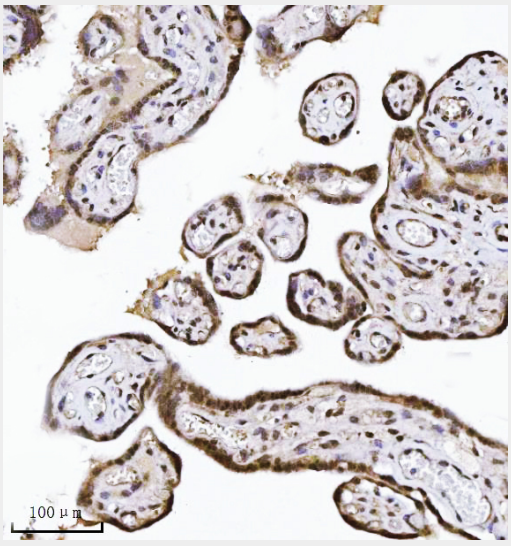

Figure 2. IHC analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
NDFIP2 was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-NDFIP2 Antibody (M08384) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB

as the chromogen.

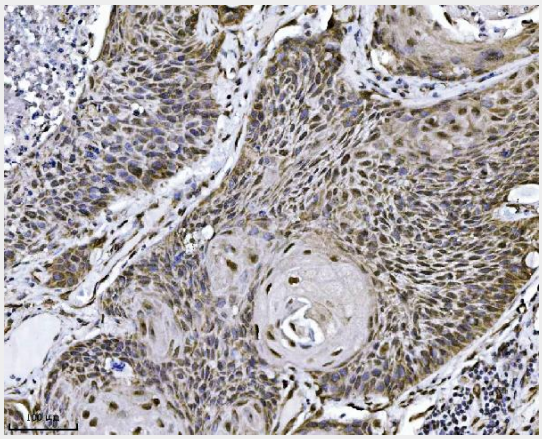

Figure 3. IHC analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
NDFIP2 was detected in a paraffin-embedded section of human squamous cell lung carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-NDFIP2 Antibody (M08384) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

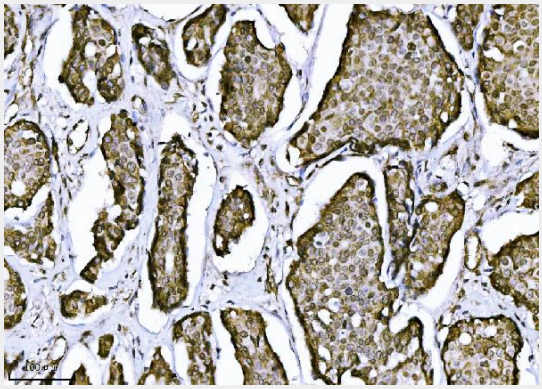

Figure 4. IHC analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
NDFIP2 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-NDFIP2 Antibody (M08384) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

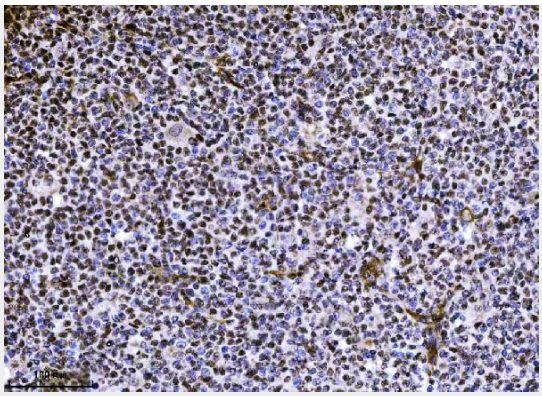

Figure 5. IHC analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
NDFIP2 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-NDFIP2 Antibody (M08384) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

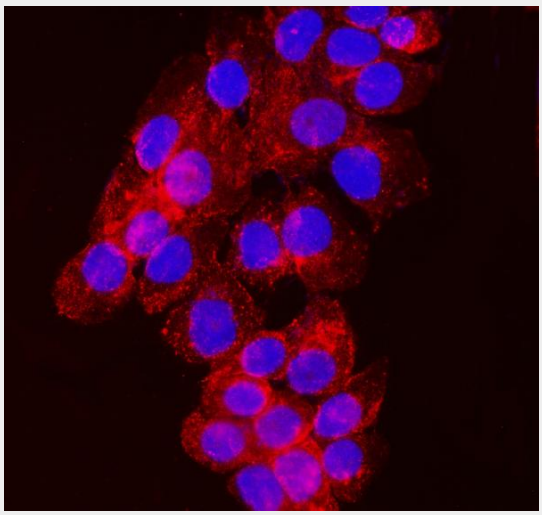

Figure 6. IF analysis of NDFIP2 using anti-NDFIP2 antibody (M08384).
NDFIP2 was detected in an immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-NDFIP2 Antibody (M08384) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) 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.

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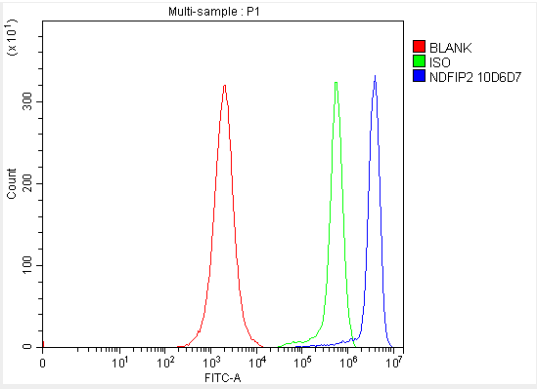

Figure 7. Flow Cytometry analysis of JK cells using anti-NDFIP2 antibody (M08384).
Overlay histogram showing JK cells stained with M08384 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-NDFIP2 Antibody (M08384, 1 µg/1x10^6 cells) for 30 min at 20°C. 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.

Anti-NDFIP2 Antibody Picoband™ (monoclonal, 10D6D7) - Background

NEDD4 family-interacting protein 2 is a protein that in humans is encoded by the NDFIP2 gene. The NEDD4 family-interacting protein 1 (NDFIP1) belongs to a small group of evolutionarily conserved proteins with three transmembrane domains and is an integral Golgi membrane protein. It is a potential target for ubiquitination by the Nedd4 family of proteins. NDFIP1 is strongly expressed in surviving neurons following acute cortical brain injury, and overexpression in cultured cortical neurons increased survival following growth factor starvation, suggesting that NDFIP1 may play a role in neuronal survival. NDFIP1 and the related protein NDFIP2 are thought to interact with and regulate multiple components of the EGF and PTEN/Akt signaling pathways. Recent studies suggest that NDFIP1 may also play a role in Th17 differentiation by limiting the production of proinflammatory cytokines.