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Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13)

Catalog # ABO15044

Specification

Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	O15357
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13) . 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 500ug/ml.

Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - Additional Information

Gene ID 3636

Other Names
Phosphatidylinositol 3, 4, 5-trisphosphate 5-phosphatase 2, 3.1.3.86, Inositol polyphosphate phosphatase-like protein 1, INPPL-1, Protein 51C, SH2 domain-containing inositol 5'-phosphatase 2 {ECO:0000312|HGNC:HGNC:6080}, SH2 domain-containing inositol phosphatase 2, SHIP-2, INPPL1 (HGNC:6080)

Calculated MW
150 kDa KDa

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

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

Immunogen
E. coli-derived human INPPL1 recombinant protein (Position: R1172-K1258).

Purification
Immunogen affinity purified.

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.

Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - Protein Information

Name INPPL1 (HGNC:6080)

Function
Phosphatidylinositol (PtdIns) phosphatase that specifically hydrolyzes the 5-phosphate of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3) to produce PtdIns(3,4)P2, thereby negatively regulating the PI3K (phosphoinositide 3-kinase) pathways (PubMed:16824732). Required for correct mitotic spindle orientation and therefore progression of mitosis (By similarity). Plays a central role in regulation of PI3K-dependent insulin signaling, although the precise molecular mechanisms and signaling pathways remain unclear (PubMed:9660833). While overexpression reduces both insulin-stimulated MAP kinase and Akt activation, its absence does not affect insulin signaling or GLUT4 trafficking (By similarity). Confers resistance to dietary obesity (By similarity). May act by regulating AKT2, but not AKT1, phosphorylation at the plasma membrane (By similarity). Part of a signaling pathway that regulates actin cytoskeleton remodeling (PubMed:11739414, PubMed:12676785). Required for the maintenance and dynamic remodeling of actin structures as well as in endocytosis, having a major impact on ligand-induced EGFR internalization and degradation (PubMed:15668240). Participates in regulation of cortical and submembraneous actin by hydrolyzing PtdIns(3,4,5)P3 thereby regulating membrane ruffling (PubMed:21624956). Regulates cell adhesion and cell spreading (PubMed:12235291). Required for HGF-mediated lamellipodium formation, cell scattering and spreading (PubMed:15735664). Acts as a negative regulator of EPHA2 receptor endocytosis by inhibiting via PI3K-dependent Rac1 activation (PubMed:17135240). Acts as a regulator of neuritogenesis by regulating PtdIns(3,4,5)P3 level and is required to form an initial protrusive pattern, and later, maintain proper neurite outgrowth (By similarity). Acts as a negative regulator of the FC-gamma-RIIA receptor (FCGR2A) (PubMed:12690104). Mediates signaling from the FC-gamma-RIIB receptor (FCGR2B), playing a central role in terminating signal transduction from activating immune/hematopoietic cell receptor systems (PubMed:11016922). Involved in EGF signaling pathway (PubMed:11349134). Upon stimulation by EGF, it is recruited by EGFR and dephosphorylates PtdIns(3,4,5)P3 (PubMed:11349134). Plays a negative role in regulating the PI3K-PKB pathway, possibly by inhibiting PKB activity (PubMed:11349134). Down-regulates Fc-gamma-R-mediated phagocytosis in macrophages independently of INPP5D/SHIP1 (By similarity). In macrophages, down-regulates NF-kappa-B-dependent gene transcription by regulating macrophage colony-stimulating factor (M-CSF)-induced signaling (By similarity). Plays a role in the localization of AURKA and NEDD9/HEF1 to the basolateral membrane at interphase in polarized cysts, thereby mediates cell cycle homeostasis, cell polarization and cilia assembly (By similarity). Additionally promotion of cilia growth is also facilitated by hydrolysis of (PtdIns(3,4,5)P3) to PtdIns(3,4)P2 (By similarity). Promotes formation of apical membrane-initiation sites during the initial stages of lumen formation via Rho family-induced actin filament organization and CTNNB1 localization to cell-cell contacts

(By similarity). May also hydrolyze PtdIns(1,3,4,5)P4, and could thus affect the levels of the higher inositol polyphosphates like InsP6. Involved in endochondral ossification (PubMed:23273569).

Cellular Location
Cytoplasm, cytosol. Cytoplasm, cytoskeleton. Membrane; Peripheral membrane protein. Cell projection, filopodium. Cell projection, lamellipodium. Basal cell membrane {ECO:0000250|UniProtKB:F1PNY0}. Nucleus {ECO:0000250|UniProtKB:D7PF45} Nucleus speckle {ECO:0000250|UniProtKB:D7PF45}. Cytoplasm, cytoskeleton, spindle pole {ECO:0000250|UniProtKB:F1PNY0} Note=Translocates to membrane ruffles when activated, translocation is probably due to different mechanisms depending on the stimulus and cell type (PubMed:11739414). Partly translocated via its SH2 domain which mediates interaction with tyrosine phosphorylated receptors such as the FC-gamma-RIIB receptor (FCGR2B). Tyrosine phosphorylation may also participate in membrane localization. Insulin specifically stimulates its redistribution from the cytosol to the plasma membrane. Recruited to the membrane following M-CSF stimulation. In activated spreading platelets, localizes with actin at filopodia, lamellipodia and the central actin ring.

Tissue Location
Widely expressed, most prominently in skeletal muscle, heart and brain. Present in platelets. Expressed in transformed myeloid cells and in primary macrophages, but not in peripheral blood monocytes.

Anti-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - 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-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - Images

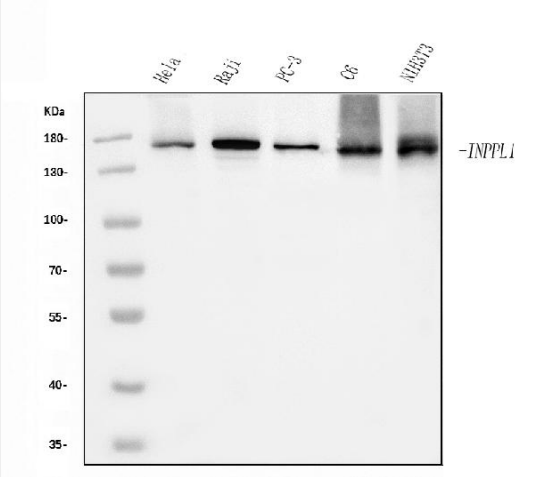

Figure 1. Western blot analysis of INPPL1 using anti-INPPL1 antibody (M01790).

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 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human Raji whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: rat C6 whole cell lysates,
Lane 5: mouse NIH/3T3 whole cell 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-INPPL1 antigen affinity purified monoclonal antibody (Catalog # M01790) 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 INPPL1 at approximately 150KD. The expected band size for INPPL1 is at 150KD.

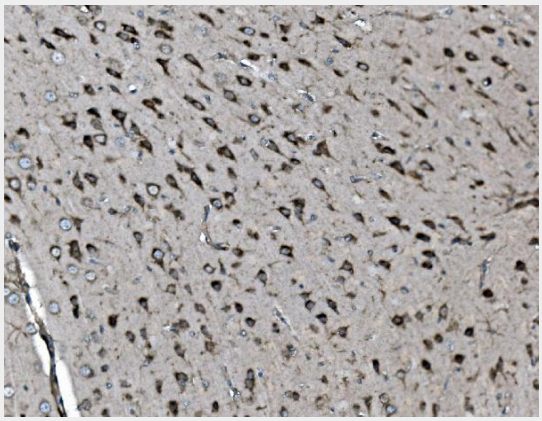

Figure 2. IHC analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-INPPL1 Antibody (M01790) 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.

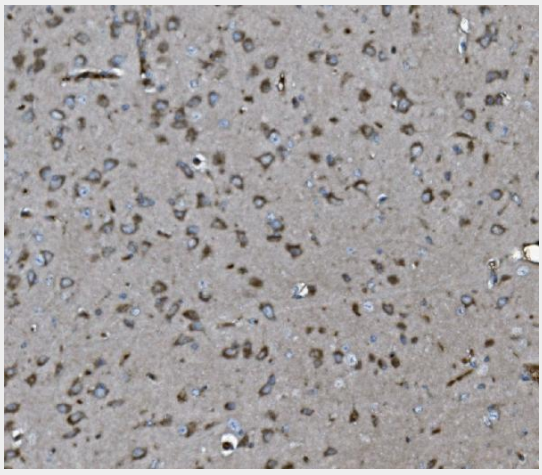

Figure 3. IHC analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was

blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-INPPL1 Antibody (M01790) 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.

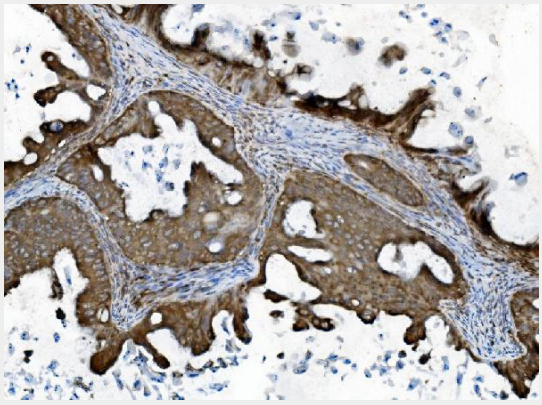

Figure 4. IHC analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-INPPL1 Antibody (M01790) 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.

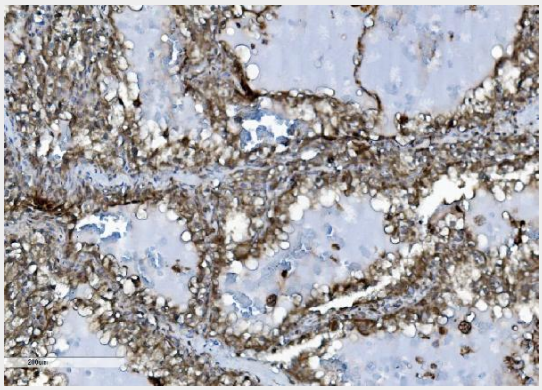

Figure 5. IHC analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-INPPL1 Antibody (M01790) 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.

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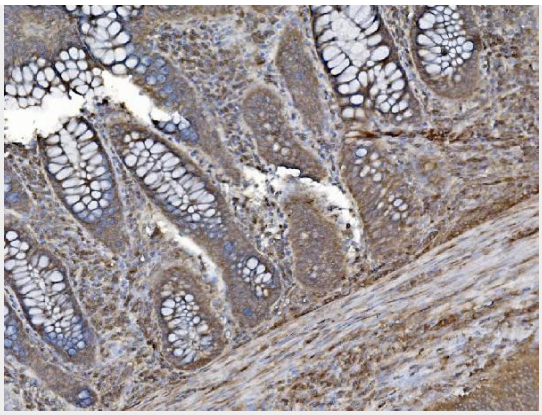

Figure 6. IHC analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-INPPL1 Antibody (M01790) 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.

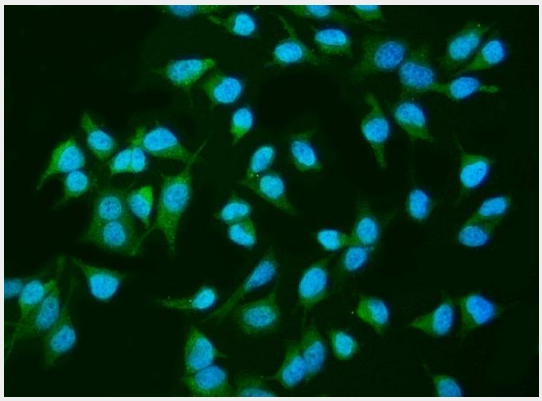

Figure 7. IF analysis of INPPL1 using anti-INPPL1 antibody (M01790).
INPPL1 was detected in immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-INPPL1 Antibody (M01790) 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.

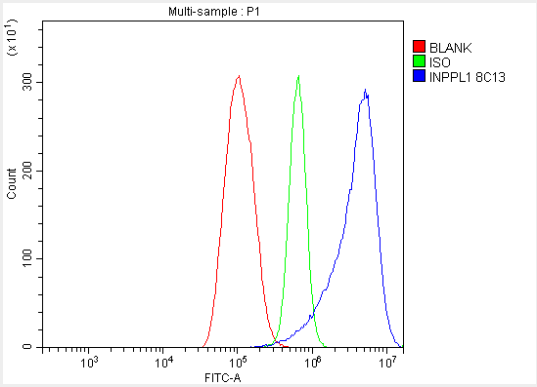

Figure 8. Flow Cytometry analysis of THP-1 cells using anti-INPPL1 antibody (M01790).
Overlay histogram showing THP-1 cells stained with M01790 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-INPPL1 Antibody (M01790, 1 µg/1x10^6 cells) for 30 min at 20°C. 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-INPPL1 Antibody Picoband™ (monoclonal, 8C13) - Background

SH2-domain containing Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 2 is an enzyme that in humans is encoded by the INPPL1 gene. The protein encoded by this gene is an SH2-containing 5'-inositol phosphatase that is involved in the regulation of insulin function. The encoded protein also plays a role in the regulation of epidermal growth factor receptor turnover and actin remodelling. Additionally, this gene supports metastatic growth in breast cancer and is a valuable biomarker for breast cancer.