

Anti-Optineurin/OPTN Antibody Picoband™ (monoclonal, 3D8)

Catalog # ABO14827

Product Information

Application	WB, FC
Primary Accession	Q96CV9
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Optineurin/OPTN Antibody Picoband™ (monoclonal, 3D8) . Tested in Flow Cytometry, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	10133
Other Names	Optineurin, E3-14.7K-interacting protein, FIP-2, Huntingtin yeast partner L, Huntingtin-interacting protein 7, HIP-7, Huntingtin-interacting protein L, NEMO-related protein, Optic neuropathy-inducing protein, Transcription factor IIIA-interacting protein, TFIIIA-IntP, OPTN
Calculated MW	65922
Application Details	Western blot, 0.1-0.5 µg/ml Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Golgi apparatus. Trans-Golgi network. Recycling endosome. Perinuclear region. Autophagosome. Cytoplasmic vesicle.
Tissue Specificity	Present in aqueous humor of the eye. Highly expressed in trabecular meshwork. Expressed nonpigmented ciliary epithelium, retina, brain, adrenal cortex, fetus, lymphocyte, fibroblast, skeletal muscle, heart, liver, brain and placenta.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 3D8

Immunogen	E.coli-derived human Optineurin recombinant protein (Position: R241-I577). Human Optineurin shares 82% amino acid (aa) sequence identity with both mouse and rat Optineurin.
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	OPTN
Function	Plays an important role in the maintenance of the Golgi complex, in membrane trafficking, in exocytosis, through its interaction with myosin VI and Rab8 (PubMed: 27534431). Links myosin VI to the Golgi complex and plays an important role in Golgi ribbon formation (PubMed: 27534431). Plays a role in the activation of innate immune response during viral infection. Mechanistically, recruits TBK1 at the Golgi apparatus, promoting its trans-phosphorylation after RLR or TLR3 stimulation (PubMed: 27538435). In turn, activated TBK1 phosphorylates its downstream partner IRF3 to produce IFN-beta/IFNB1. Plays a neuroprotective role in the eye and optic nerve. May act by regulating membrane trafficking and cellular morphogenesis via a complex that contains Rab8 and huntingtin (HD). Mediates the interaction of Rab8 with the probable GTPase-activating protein TBC1D17 during Rab8-mediated endocytic trafficking, such as that of transferrin receptor (TFRC/TfR); regulates Rab8 recruitment to tubules emanating from the endocytic recycling compartment (PubMed: 22854040). Autophagy receptor that interacts directly with both the cargo to become degraded and an autophagy modifier of the MAP1 LC3 family; targets ubiquitin- coated bacteria (xenophagy), such as cytoplasmic Salmonella enterica, and appears to function in the same pathway as SQSTM1 and CALCOCO2/NDP52.
Cellular Location	Cytoplasm, perinuclear region. Golgi apparatus. Golgi apparatus, trans-Golgi network Cytoplasmic vesicle, autophagosome. Cytoplasmic vesicle. Recycling endosome. Note=Found in the perinuclear region and associates with the Golgi apparatus (PubMed: 27534431) Colocalizes with MYO6 and RAB8 at the Golgi complex and in vesicular structures close to the plasma membrane. Localizes to LC3-positive cytoplasmic vesicles upon induction of autophagy
Tissue Location	Present in aqueous humor of the eye (at protein level). Expressed in the trabecular meshwork (at protein level) (PubMed: 11834836 , PubMed: 12379221 , PubMed: 12646749). Expressed in nonpigmented ciliary epithelium (at protein level) (PubMed: 11834836) Expressed at high levels in skeletal muscle, also detected in heart, brain, pancreas, kidney, placenta and liver (PubMed: 9488477). Expressed in dermal fibroblasts (at protein level) (PubMed: 11834836)

Background

OPTN is also known as NRP, FIP2 or HYPL. This gene encodes the coiled-coil containing protein optineurin. Optineurin may play a role in normal-tension glaucoma and adult-onset primary open angle glaucoma. Optineurin interacts with adenovirus E3-14.7K protein and may utilize tumor necrosis factor-alpha or Fas-ligand pathways to mediate apoptosis, inflammation or vasoconstriction. Optineurin may also function in cellular morphogenesis and membrane trafficking, vesicle trafficking, and transcription activation through its interactions with the RAB8, huntingtin, and transcription factor IIIA proteins. Alternative splicing results in multiple transcript variants encoding the same protein.

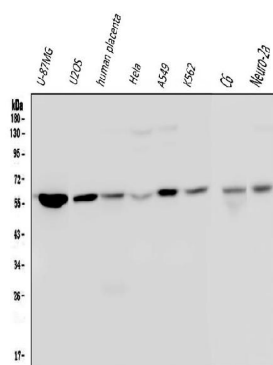


Figure 1. Western blot analysis of Optineurin/OPTN using anti-Optineurin/OPTN antibody (M00952).

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 U-87MG whole cell lysates;

Lane 2: human U20S whole cell lysates;

Lane 3: human placenta tissue lysates;

Lane 4: human Hela whole cell lysates;

Lane 5: human A549 whole cell lysates;

Lane 6: human K562 whole cell lysates;

Lane 7: rat C6 whole cell lysates;

Lane 8: mouse Neuro-2a 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-Optineurin/OPTN antigen affinity purified

monoclonal antibody (Catalog # M00952) 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 Optineurin/OPTN at approximately

66KD. The expected band size for Optineurin/OPTN is at 66KD.

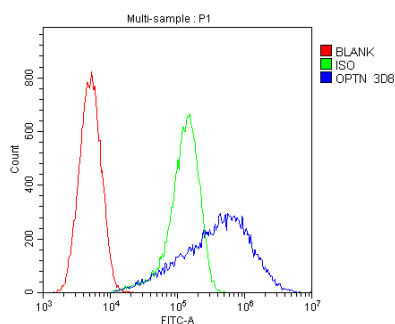


Figure 2. Flow Cytometry analysis of U20S cells using anti-Optineurin/OPTN antibody (M00952).

Overlay histogram showing U20S cells stained with M00952 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Optineurin/OPTN Antibody (M00952, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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