10320 Camino Santa Fe, Suite G
San Diego, CA 92121
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Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10)

Catalog # ABO14908

Specification

Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	Q8WUM4
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-ALIX/PDCD6IP Antibody Picoband™ (monoclonal, 14D10) . 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 µg/ml.

Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Additional Information

Gene ID 10015

Other Names
Programmed cell death 6-interacting protein, PDCD6-interacting protein, ALG-2-interacting protein 1, ALG-2-interacting protein X, Hp95, PDCD6IP (HGNC:8766), AIP1, ALIX, KIAA1375

Calculated MW
96-100 kDa KDa

Application Details
"Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat, By Heat
 Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6, Human
"

Subcellular Localization
exosome; cytosol; centrosome; Melanosome; tight junction; Midbody ring

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.

Immunogen
E.coli-derived human PDCD6IP recombinant protein (Position: A2-D330). Human PDCD6IP shares 96.7% and 95.2% amino acid (aa) sequence identity with mouse and rat PDCD6IP, respectively.

Purification
Immunogen affinity purified.

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.**

Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Protein Information

Name PDCD6IP (HGNC:8766)

Synonyms AIP1, ALIX, KIAA1375

Function
Multifunctional protein involved in endocytosis, multivesicular body biogenesis, membrane repair, cytokinesis, apoptosis and maintenance of tight junction integrity. Class E VPS protein involved in concentration and sorting of cargo proteins of the multivesicular body (MVB) for incorporation into intralumenal vesicles (ILVs) that are generated by invagination and scission from the limiting membrane of the endosome. Binds to the phospholipid lysobisphosphatidic acid (LBPA) which is abundant in MVBs internal membranes. The MVB pathway requires the sequential function of ESCRT-O, -I,-II and -III complexes (PubMed:14739459). The ESCRT machinery also functions in topologically equivalent membrane fission events, such as the terminal stages of cytokinesis (PubMed:17556548, PubMed:17853893). Adapter for a subset of ESCRT-III proteins, such as CHMP4, to function at distinct membranes. Required for completion of cytokinesis (PubMed:17556548, PubMed:17853893, PubMed:18641129). May play a role in the regulation of both apoptosis and cell proliferation. Regulates exosome biogenesis in concert with SDC1/4 and SDCBP (PubMed:22660413). By interacting with F-actin, PARD3 and TJP1 secures the proper assembly and positioning of actomyosin-tight junction complex at the apical sides of adjacent epithelial cells that defines a spatial membrane domain essential for the maintenance of epithelial cell polarity and barrier (By similarity).

Cellular Location
Cytoplasm, cytosol {ECO:0000250|UniProtKB:Q9QZA2}. Melanosome. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Secreted, extracellular exosome. Cell junction, tight junction {ECO:0000250|UniProtKB:Q9WU78}. Midbody, Midbody ring Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Colocalized with CEP55 at centrosomes of non-dividing cells. Component of the actomyosin-tight junction complex (By similarity). PDCD6IP targeting to the midbody requires the interaction with CEP55 (PubMed:18641129). {ECO:0000250|UniProtKB:Q9QZA2, ECO:0000250|UniProtKB:Q9WU78, ECO:0000269|PubMed:17081065, ECO:0000269|PubMed:17556548, ECO:0000269|PubMed:17853893, ECO:0000269|PubMed:18641129}

Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Protocols

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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-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Images

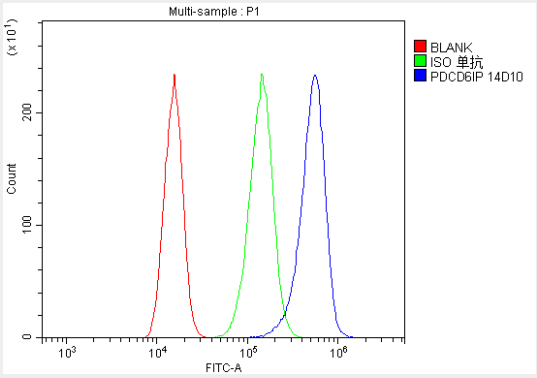

Figure 9. Flow Cytometry analysis of A431 cells using anti-PDCD6IPantibody (M01751-1).
Overlay histogram showing A431 cells stained with M01751-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PDCD6IP Antibody (M01751-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight488 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.

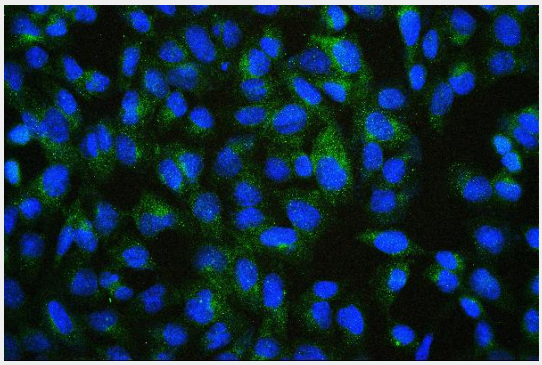

Figure 8. IF analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-PDCD6IP Antibody (M01751-1) overnight at 4°C. DyLight488 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.

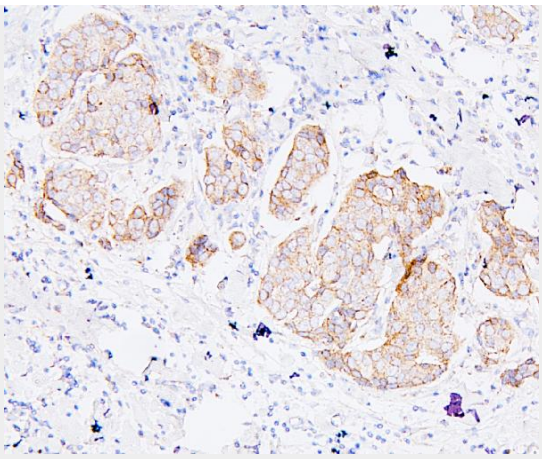

Figure 2. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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.

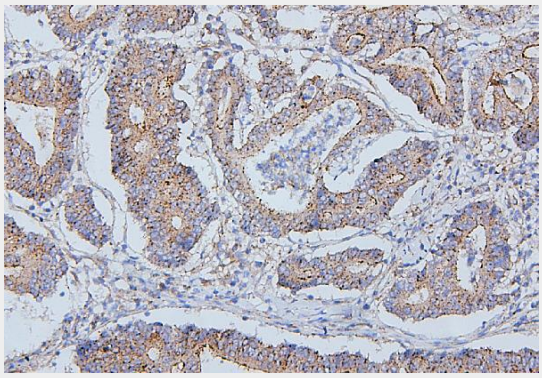

Figure 3. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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.

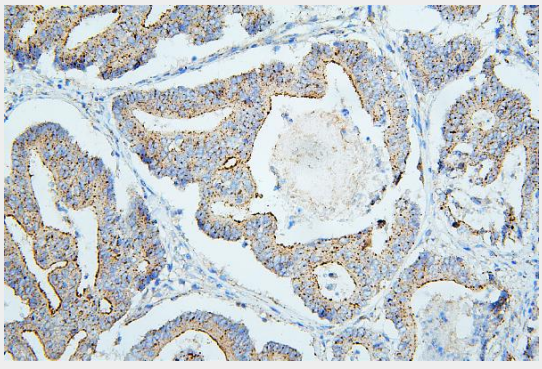

Figure 4. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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.

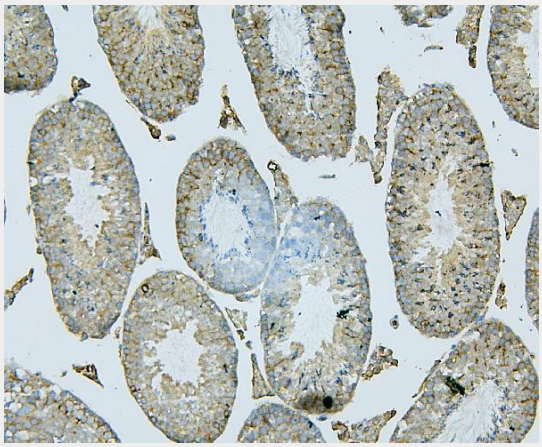

Figure 5. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of mouse testis tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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.

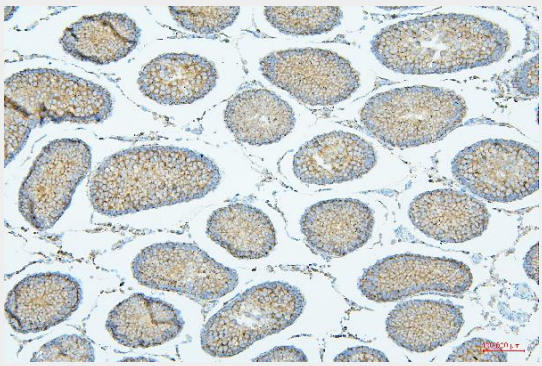

Figure 6. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of rat testis tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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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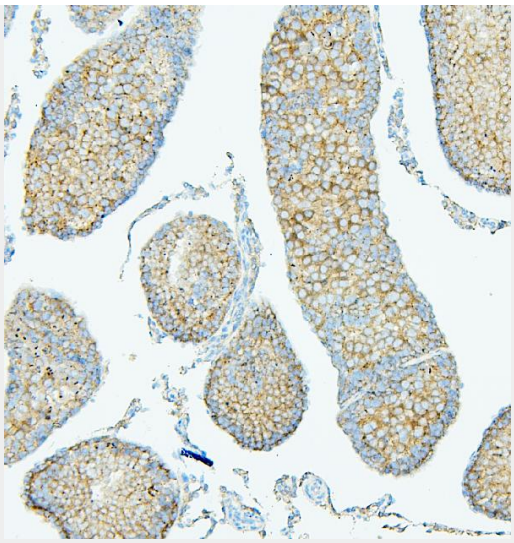

Figure 7. IHC analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
PDCD6IP was detected in paraffin-embedded section of rat testis tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PDCD6IP Antibody (M01751-1) 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 1. Western blot analysis of PDCD6IP using anti-PDCD6IP antibody (M01751-1).
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 Hela whole cell lysates
Lane 2: human HepG2 whole cell lysates
Lane 3: human Jurkat whole cell lysates
Lane 4: human PANC-1 whole cell lysates
Lane 5: human K562 whole cell lysates
Lane 6: human SW579 whole cell lysates
Lane 7: rat RH35 whole cell lysates
Lane 8: mouse NIH3T3 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-PDCD6IP antigen affinity purified monoclonal antibody (Catalog # M01751-1) 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 PDCD6IP at approximately 96-100KD. The expected band size for PDCD6IP is at 96KD.

Anti-ALIX/PDCD6IP Antibody Picoband™(monoclonal, 14D10) - Background

Programmed cell death 6-interacting protein is a protein that in humans is encoded by the PDCD6IP gene. This gene encodes a protein that functions within the ESCRT pathway in the abscission stage of cytokinesis, in intralumenal endosomal vesicle formation, and in enveloped virus budding. Studies using mouse cells have shown that overexpression of this protein can block apoptosis. In addition, the product of this gene binds to the product of the PDCD6 gene, a protein required for apoptosis, in a calcium-dependent manner. This gene product also binds to endophilins, proteins that regulate membrane shape during endocytosis. Overexpression of this gene product and endophilins results in cytoplasmic vacuolization, which may be partly responsible for the protection against cell death. Several alternatively spliced transcript variants encoding different isoforms have been found for this gene.