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Anti-PPID Antibody Picoband™ (monoclonal, 5A6)

Catalog # ABO14819

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q08752
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PPID Antibody Picoband™ (monoclonal, 5A6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.

Additional Information

Gene ID	5481
Other Names	Peptidyl-prolyl cis-trans isomerase D, PPIase D, 5.2.1.8, 40 kDa peptidyl-prolyl cis-trans isomerase, Cyclophilin-40, CYP-40, Cyclophilin-related protein, Rotamase D, PPID (HGNC:9257), CYP40, CYPD
Calculated MW	40764
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	Widely expressed.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 5A6
Immunogen	E. coli-derived human PPID recombinant protein (Position: N306-A370).
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	PPID (HGNC:9257)
Synonyms	CYP40, CYPD
Function	PPIase that catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides and may therefore assist protein folding (PubMed:11350175, PubMed:20676357). Proposed to act as a co- chaperone in HSP90 complexes such as in unligated steroid receptors heterocomplexes. Different co-chaperones seem to compete for association with HSP90 thus establishing distinct HSP90-co-chaperone- receptor complexes with the potential to exert tissue-specific receptor activity control. May have a preference for estrogen receptor complexes and is not found in glucocorticoid receptor complexes. May be involved in cytoplasmic dynein-dependent movement of the receptor from the cytoplasm to the nucleus. May regulate MYB by inhibiting its DNA- binding activity. Involved in regulation of AHR signaling by promoting the formation of the AHR:ARNT dimer; the function is independent of HSP90 but requires the chaperone activity. Involved in regulation of UV radiation-induced apoptosis. Promotes cell viability in anaplastic lymphoma kinase-positive anaplastic large-cell lymphoma (ALK+ ALCL) cell lines.
Cellular Location	Cytoplasm. Nucleus, nucleolus. Nucleus, nucleoplasm
Tissue Location	Widely expressed.

Background

Cyclophilin D, Peptidylprolyl isomerase D, also known as PPID, is an enzyme which in humans is encoded by the PPID gene. The protein encoded by this gene is a member of the peptidyl-prolyl cis-trans isomerase (PPIase) family. The Cyclophilin D (PPID) gene contains 10 exons and spans 14.2 kb of genomic DNA. By fluorescence in situ hybridization, the PPID gene is mapped to chromosome 4q31.3. PPIases catalyze the cis-trans isomerization of proline imidic peptide bonds in oligopeptides and accelerate the folding of proteins. This protein has been shown to possess PPIase activity and, similar to other family members, can bind to the immunosuppressant ciclosporin.

Images

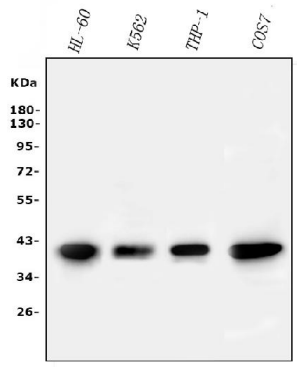


Figure 1. Western blot analysis of PPID using anti-PPID antibody (M02424).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: HL-60 whole cell lysates,
Lane 2: K562 whole cell lysates,
Lane 3: THP-1 whole cell lysates,
Lane 4: COS-7 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-PPID antigen affinity purified monoclonal antibody (Catalog # M02424) 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 PPID at approximately 41KD. The expected band size for PPID is at 41KD.

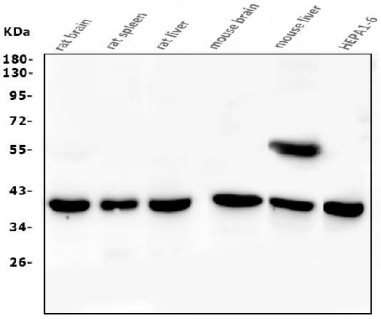


Figure 2. Western blot analysis of PPID using anti-PPID antibody (M02424).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat brain tissue lysates,
Lane 2: rat spleen tissue lysates,
Lane 3: rat liver tissue lysates,
Lane 4: mouse brain tissue lysates,
Lane 5: mouse liver tissue lysates,
Lane 6: HEPA1-6 whole cell lysate.
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-PPID antigen affinity purified monoclonal antibody (Catalog # M02424) 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 PPID at approximately 41KD. The expected band size for PPID is at 41KD.

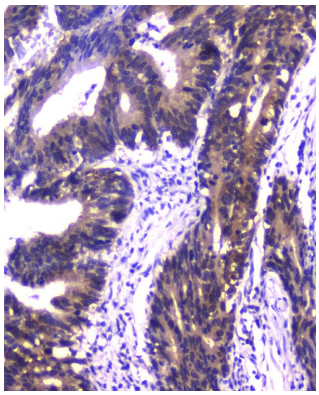


Figure 3. IHC analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PPID Antibody (M02424) 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.

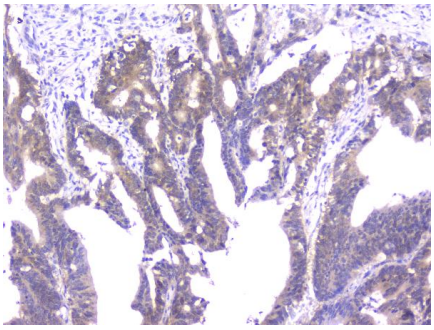


Figure 4. IHC analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PPID Antibody (M02424) 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.

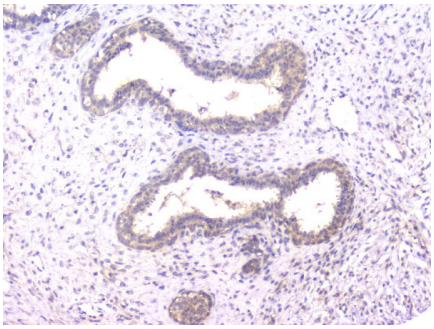


Figure 5. IHC analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PPID Antibody (M02424) 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.

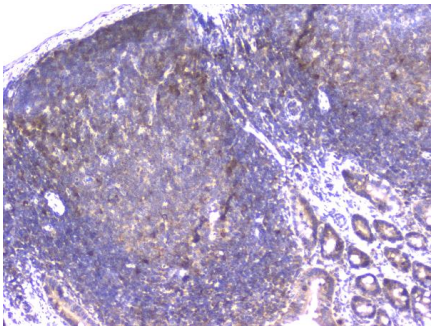


Figure 6. IHC analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PPID Antibody (M02424) 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.

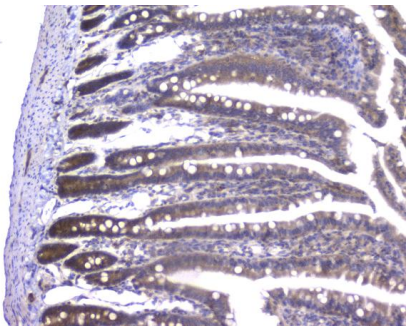


Figure 7. IHC analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PPID Antibody (M02424) 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.

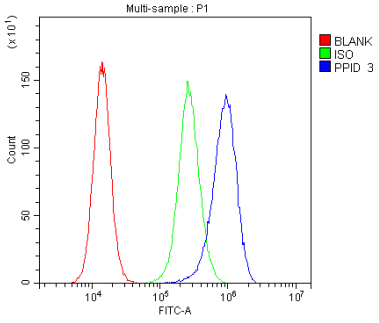


Figure 8. Flow Cytometry analysis of A431 cells using anti-PPID antibody (M02424).
Overlay histogram showing A431 cells stained with M02424 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PPID Antibody (M02424,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight488 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.

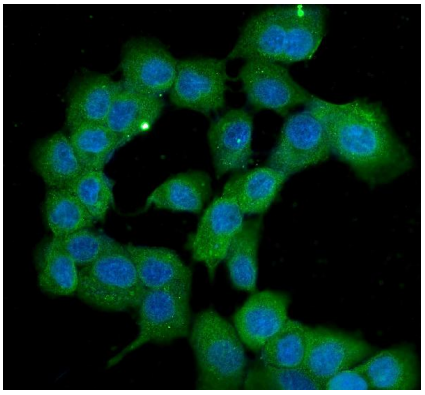


Figure 9. IF analysis of PPID using anti-PPID antibody (M02424).
PPID was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-PPID Antibody (M02424) 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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.