

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

Catalog Number: M02424

About PPID

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.

Overview

Product Name	Anti-PPID Antibody Picoband™ (monoclonal, 5A6)
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-PPID Antibody Picoband™ (monoclonal, 5A6) catalog # M02424. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 5A6
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	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.
Host	Mouse
Uniprot ID	Q08752

Technical Details

Immunogen	E. coli-derived human PPID recombinant protein (Position: N306-A370).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.

Suggested Dilutions

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.1-0.5ug/ml

Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml

Immunocytochemistry/Immunofluorescence, 5ug/ml

Flow Cytometry, 1-3ug/1x10⁶ cells

Anti-PPID Antibody Picoband™ (monoclonal, 5A6) (M02424) Images

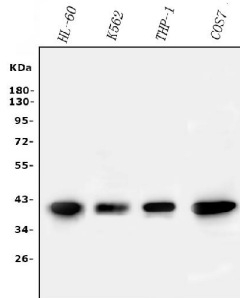


Figure 1. Western blot analysis of PPID using anti-PPID antibody (M02424).

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 ug/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.

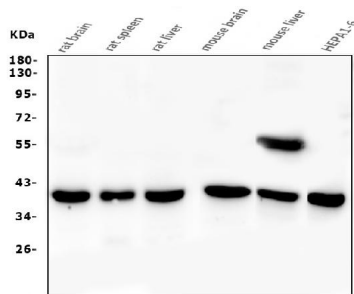


Figure 2. Western blot analysis of PPID using anti-PPID antibody (M02424).

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 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed

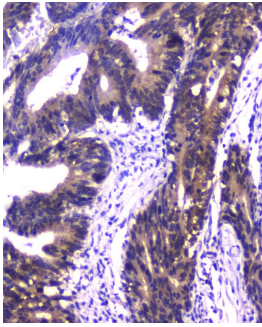
with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed

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 1ug/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

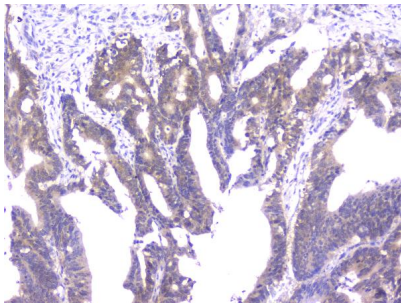


Figure 4. IHC analysis of PPID using anti-PPID antibody (M02424).

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 1ug/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

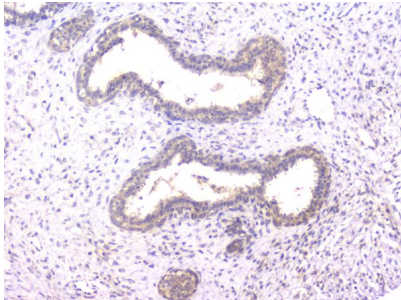


Figure 5. IHC analysis of PPID using anti-PPID antibody (M02424).

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 1ug/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

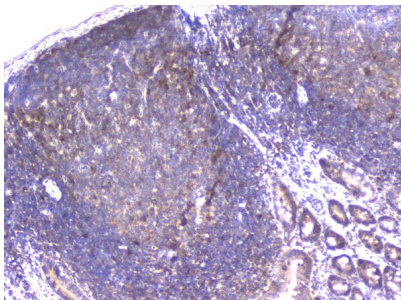
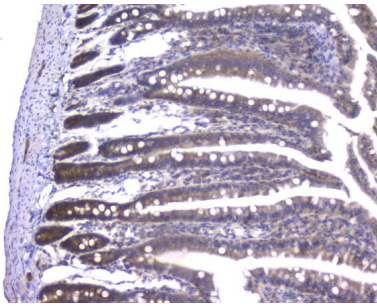


Figure 6. IHC analysis of PPID using anti-PPID antibody (M02424).

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 1ug/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 Streptavidin-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 1ug/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

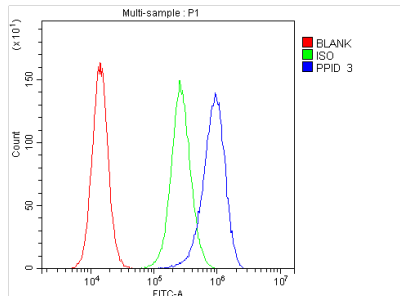


Figure 8. Flow Cytometry analysis of A431 cells using anti-PPID antibody (M02424). Overlay histogram showing A431 cells stained with M02424 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PPID Antibody (M02424, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

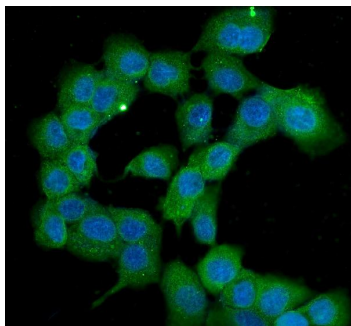


Figure 9. IF analysis of PPID using anti-PPID antibody (M02424). PPID was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL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

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