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Anti-PDE6 beta/PDE6B Antibody Picoband™ (monoclonal, 8I2F10)

Catalog # ABO15091

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P35913
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PDE6 beta/PDE6B Antibody Picoband™ (monoclonal, 8I2F10) . Tested in FCM, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	5158
Other Names	Rod cGMP-specific 3', 5'-cyclic phosphodiesterase subunit beta, GMP-PDE beta, 3.1.4.35, PDE6B (HGNC:8786), PDEB
Calculated MW	98336
Application Details	Western blot, 0.25-0.5 □g/ml, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Rat Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 8I2F10
Immunogen	E.coli-derived human PDE6 beta/PDE6B recombinant protein (Position: K25-Q237).
Purification	Immunogen affinity purified.
Storage	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 freezing and thawing.

Protein Information

Name	PDE6B (HGNC:8786)
Synonyms	PDEB
Function	Catalytic beta subunit of the rod-specific cGMP phosphodiesterase (PDE6) complex, which hydrolyzes 3',5'-cyclic GMP in the phototransduction cascade. The PDE6 holoenzyme consists of two catalytic subunits (PDE6A and PDE6B) and two inhibitory gamma subunits (PDE6G) (PubMed:20940301). Light-activated GNAT1 relieves gamma subunit-mediated inhibition, enabling the catalytic subunits to hydrolyze cGMP and thereby mediate visual signal transduction and amplification (PubMed:8394174). Decreased cytosolic cGMP levels result in closure of cGMP-gated cation channels at the plasma membrane, leading to rod photoreceptor hyperpolarization (Probable). Involved in retinal circadian rhythm photoentrainment via modulation of UVA and orange light-induced phase-shift of the retina clock (By similarity).
Cellular Location	Photoreceptor outer segment membrane; Lipid-anchor {ECO:0000250|UniProtKB:D3ZDI8}. Membrane {ECO:0000250|UniProtKB:P23439}; Lipid-anchor {ECO:0000250|UniProtKB:D3ZDI8}

Background

Photon absorption triggers a signaling cascade in rod photoreceptors that activates cGMP phosphodiesterase (PDE), resulting in the rapid hydrolysis of cGMP, closure of cGMP-gated cation channels, and hyperpolarization of the cell. PDE is a peripheral membrane heterotrimeric enzyme made up of alpha, beta, and gamma subunits. This gene encodes the beta subunit. Mutations in this gene result in retinitis pigmentosa and autosomal dominant congenital stationary night blindness. Multiple transcript variants encoding different isoforms have been found for this gene.

Images

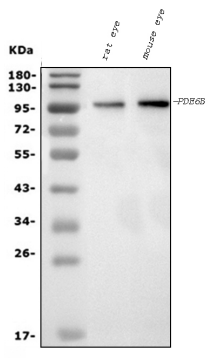


Figure 1. Western blot analysis of PDE6 beta/PDE6B using anti-PDE6 beta/PDE6B antibody (M02659). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat eye tissue lysates,
Lane 2: mouse eye tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-PDE6 beta/PDE6B antigen affinity purified monoclonal antibody (Catalog # M02659) 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 PDE6 beta/PDE6B at

approximately 98 kDa. The expected band size for PDE6 beta/PDE6B is at 98 kDa.

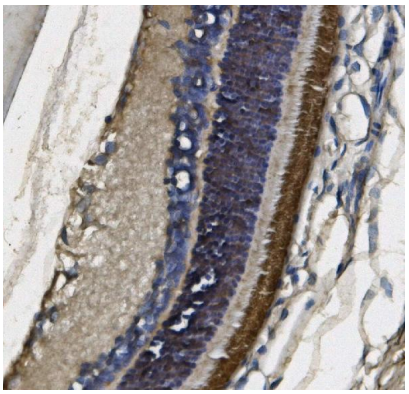


Figure 2. IHC analysis of PDE6 beta/PDE6B using anti-PDE6 beta/PDE6B antibody (M02659).
PDE6 beta/PDE6B was detected in a paraffin-embedded section of rat eye ball tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PDE6 beta/PDE6B Antibody (M02659) 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.

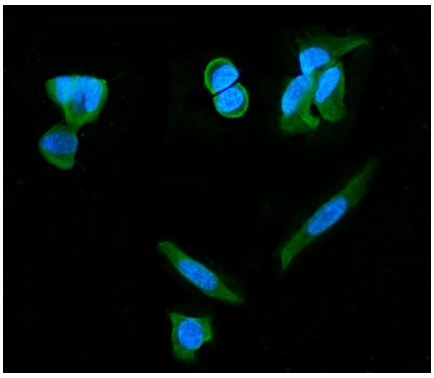


Figure 3. IF analysis of PDE6 beta/PDE6B using anti-PDE6 beta/PDE6B antibody (M02659).
PDE6 beta/PDE6B was detected in an immunocytochemical section of SH-SY5Y cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-PDE6 beta/PDE6B Antibody (M02659) 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.

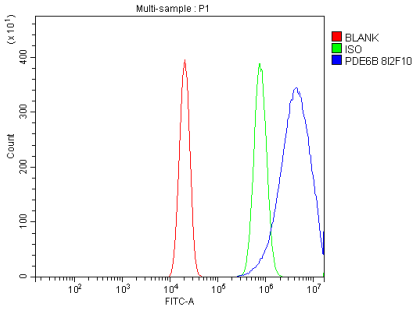


Figure 4. Flow Cytometry analysis of U20S cells using anti-PDE6 beta/PDE6B antibody (M02659).
Overlay histogram showing U20S cells stained with M02659 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PDE6 beta/PDE6B Antibody (M02659, 1 µg/1x10^6 cells) 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.

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