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Anti-PP2A-alpha/PPP2CA Antibody Picoband™ (monoclonal, 3B6)

Catalog # ABO14963

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P67775
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PP2A-alpha/PPP2CA Antibody Picoband™ (monoclonal, 3B6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	5515
Other Names	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform, PP2A-alpha, 3.1.3.16, Replication protein C, RP-C, PPP2CA
Calculated MW	35594
Application Details	Western blot, 0.1-0.5 □g/ml, Human, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 4 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Clone Names	Clone: 3B6
Immunogen	E.coli-derived human PP2A-alpha recombinant protein (Position: M1-L309). Human PP2A-alpha shares 100% amino acid (aa) sequence identity with both mouse and rat PP2A-alpha.
Purification	Immunogen affinity purified.
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	PPP2CA
Function	Catalytic subunit of protein phosphatase 2A (PP2A), a serine/threonine phosphatase involved in the regulation of a wide variety of enzymes, signal transduction pathways, and cellular events (PubMed:10801873, PubMed:12473674, PubMed:17245430, PubMed:22613722, PubMed:33243860, PubMed:34004147, PubMed:9920888, PubMed:41986709). PP2A is the major phosphatase for microtubule-associated proteins (MAPs) (PubMed:22613722). PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase (PubMed:22613722). Cooperates with SGO2 to protect centromeric cohesin from separase-mediated cleavage in oocytes specifically during meiosis I (By similarity). Can dephosphorylate various proteins, such as SV40 large T antigen, AXIN1, p53/TP53, PIM3, WEE1 (PubMed:10801873, PubMed:12473674, PubMed:17245430, PubMed:9920888). Activates RAF1 by dephosphorylating it at 'Ser-259' (PubMed:10801873). Mediates dephosphorylation of WEE1, preventing its ubiquitin-mediated proteolysis, increasing WEE1 protein levels, and promoting the G2/M checkpoint (PubMed:33108758). Mediates dephosphorylation of MYC; promoting its ubiquitin-mediated proteolysis: interaction with AMBRA1 enhances interaction between PPP2CA and MYC (PubMed:25438055). Mediates dephosphorylation of FOXO3; promoting its stabilization: interaction with AMBRA1 enhances interaction between PPP2CA and FOXO3 (PubMed:30513302). Catalyzes dephosphorylation of the pyrin domain of NLRP3, promoting assembly of the NLRP3 inflammasome (By similarity). Together with RACK1 adapter, mediates dephosphorylation of AKT1 at 'Ser-473', preventing AKT1 activation and AKT-mTOR signaling pathway (By similarity). Dephosphorylation of AKT1 is essential for regulatory T-cells (Treg) homeostasis and stability (By similarity). Catalyzes dephosphorylation of PIM3, promoting PIM3 ubiquitination and proteasomal degradation (PubMed:12473674). Part of the striatin- interacting phosphatase and kinase (STRIPAK) complexes (PubMed:33633399). STRIPAK complexes have critical roles in protein (de)phosphorylation and are regulators of multiple signaling pathways including Hippo, MAPK, nuclear receptor and cytoskeleton remodeling (PubMed:33633399). Different types of STRIPAK complexes are involved in a variety of biological processes such as cell growth, differentiation, apoptosis, metabolism and immune regulation (PubMed:33633399). Key mediator of a quality checkpoint during transcription elongation as part of the Integrator-PP2A (INTAC) complex (PubMed:33243860, PubMed:34004147, PubMed:37080207). The INTAC complex drives premature transcription termination of transcripts that are unfavorably configured for transcriptional elongation: within the INTAC complex, PPP2CA catalyzes dephosphorylation of the C-terminal domain (CTD) of Pol II subunit POLR2A/RPB1 and SUPT5H/SPT5, thereby preventing transcriptional elongation (PubMed:33243860, PubMed:34004147, PubMed:37080207).
Cellular Location	Cytoplasm. Nucleus. Chromosome. Chromosome, centromere. Cytoplasm, cytoskeleton, spindle pole. Note=In prometaphase cells, but not in anaphase cells, localizes at centromeres (PubMed:16541025). During mitosis, also found at spindle poles (PubMed:16541025). Centromeric localization requires the presence of SGO2 (By similarity). Recruited to chromatin and transcription pause-release checkpoint via its association with the Integrator complex (PubMed:33243860, PubMed:34004147). {ECO:0000250|UniProtKB:P63330, ECO:0000269|PubMed:16541025, ECO:0000269|PubMed:33243860,

ECO:0000269|PubMed:34004147}

Background

The catalytic subunit of human protein phosphatase 2A (PPP2CA) encodes a 309-amino acid polypeptide. It is localized to chromosome 5. The gene (approximately 30 kbp) is composed of seven exons and six introns. It is predicted to be important for phosphatase enzymatic activity. Methylation of the C-terminal leucine residue (Leu309) of protein serine/threonine phosphatase 2A catalytic subunit (PP2AC) is known to regulate catalytic activity in vitro. Furthermore, PP2A has a fundamental role in cardiac function, and suggests that disturbances in protein phosphatase expression and activity may cause or exacerbate the course of cardiac diseases.

Images

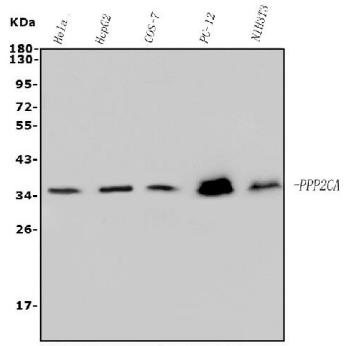

Figure 1. Western blot analysis of PP2A-alpha/PPP2CA using anti-PP2A-alpha/PPP2CA antibody (M01893-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HELA whole cell lysates,
Lane 2: human HEPG2 whole cell lysates,
Lane 3: monkey COS-7 whole cell lysates,
Lane 4: rat PC-12 whole cell lysates,
Lane 5: mouse NIH/3T3 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-PP2A-alpha/PPP2CA antigen affinity purified monoclonal antibody (Catalog # M01893-1) 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 PP2A-alpha/PPP2CA at approximately 36KD. The expected band size for PP2A-alpha/PPP2CA is at 36KD.

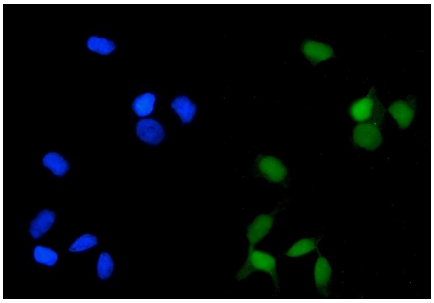

Figure 2. IF analysis of PP2A-alpha/PPP2CA using anti-PP2A-alpha/PPP2CA antibody (M01893-1). PP2A-alpha/PPP2CA was detected in immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 4 µg/mL mouse anti-PP2A-alpha/PPP2CA Antibody (M01893-1) 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.

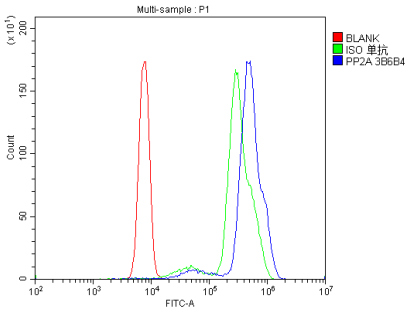

Figure 3. Flow Cytometry analysis of U937 cells using anti-PP2A-alpha/PPP2CA antibody (M01893-1). Overlay histogram showing U937 cells stained with M01893-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PP2A-alpha/PPP2CA Antibody (M01893-1, 1 µg/1x10^6 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.

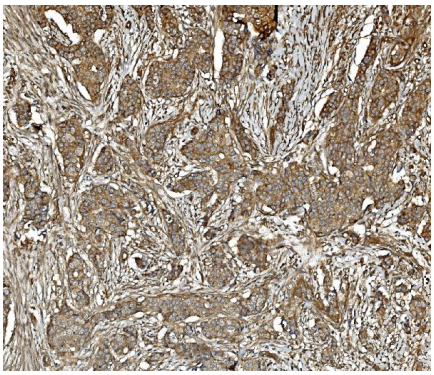

Figure 4. IHC analysis of PP2A-alpha/PPP2CA using anti-PP2A-alpha/PPP2CA antibody (M01893-1). PP2A-alpha/PPP2CA was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PP2A-alpha/PPP2CA Antibody (M01893-1) 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.

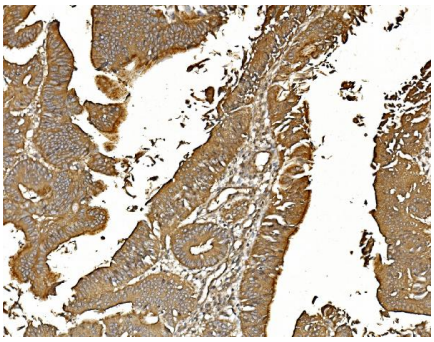

Figure 5. IHC analysis of PP2A-alpha/PPP2CA using anti-PP2A-alpha/PPP2CA antibody (M01893-1). PP2A-alpha/PPP2CA was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PP2A-alpha/PPP2CA Antibody (M01893-1) 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.

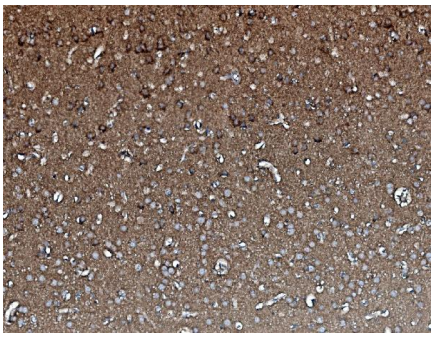

Figure 6. IHC analysis of PP2A-alpha/PPP2CA using anti-PP2A-alpha/PPP2CA antibody (M01893-1). PP2A-alpha/PPP2CA was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-PP2A-alpha/PPP2CA Antibody (M01893-1) 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.

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