abcam

Product datasheet

Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free ab235385

Recombinant RabMAb

9 Images

Overview

Product name	Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free
Description	Rabbit monoclonal [EP2102Y] to cAMP Protein Kinase Catalytic subunit - BSA and Azide free
Host species	Rabbit
Specificity	The immunogen used for this product shares 92% homology with PKA C-beta and PKA C-gamma. Cross-reactivity with these proteins have not been confirmed experimentally.
Tested applications	**Suitable for:** WB, IP, IHC-P, Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HeLa cells; Flow Cyt (intra): MCF-7 and HeLa cells; IHC-P: Rat stomach tissue, Mouse cerebrum tissue, Human testis and thyroid carcinoma tissue; WB: MCF-7, HeLa, NIH/3T3 and C6 cell lysates.
General notes	ab235385 is the carrier-free version of ab76238. Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2102Y
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab235385 in the following tested applications.

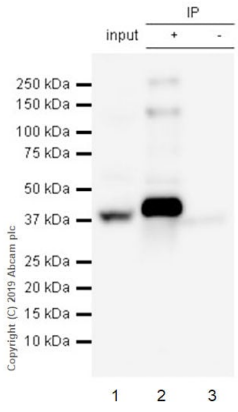
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 42 kDa (predicted molecular weight: 46 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. Use of HRP-conjugated or polymerized HRP secondary antibodies recommended, stronger signals have been found using the polymerized HRP secondary
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

Target

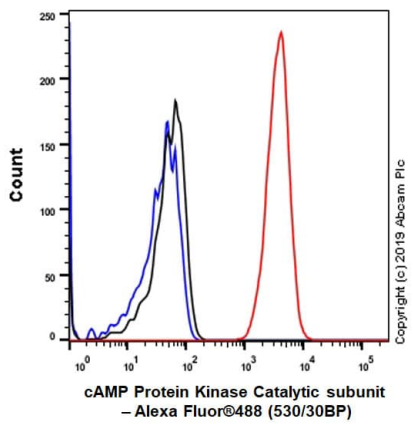
Function	Phosphorylates a large number of substrates in the cytoplasm and the nucleus. Regulates the abundance of compartmentalized pools of its regulatory subunits through phosphorylation of PJA2 which binds and ubiquitinates these subunits, leading to their subsequent proteolysis. Phosphorylates CDC25B, ABL1, NFKB1, CLDN3, PSMC5/RPT6, PJA2, RYR2, RORA and VASP. RORA is activated by phosphorylation. Required for glucose-mediated adipogenic differentiation increase and osteogenic differentiation inhibition from osteoblasts. Involved in the regulation of platelets in response to thrombin and collagen; maintains circulating platelets in a resting state by phosphorylating proteins in numerous platelet inhibitory pathways when in complex with NF-kappa-B (NFKB1 and NFKB2) and I-kappa-B-alpha (NFKBIA), but thrombin and collagen disrupt these complexes and free active PRKACA stimulates platelets and leads to platelet aggregation by phosphorylating VASP. Prevents the antiproliferative and anti-invasive effects of alpha-difluoromethylornithine in breast cancer cells when activated. RYR2 channel activity is potentiated by phosphorylation in presence of luminal Ca(2+), leading to reduced amplitude and increased frequency of store overload-induced Ca(2+) release (SOICR) characterized by an increased rate of Ca(2+) release and propagation velocity of spontaneous Ca(2+) waves, despite reduced wave amplitude and resting cytosolic Ca(2+). PSMC5/RPT6 activation by phosphorylation stimulates proteasome. Negatively regulates tight junctions (TJs) in ovarian cancer cells via CLDN3 phosphorylation. NFKB1 phosphorylation promotes NF-kappa-B p50-p50 DNA binding. Involved in embryonic development by down-regulating the Hedgehog (Hh) signaling pathway that determines embryo pattern formation and morphogenesis. Prevents meiosis resumption in prophase-arrested oocytes via CDC25B inactivation by phosphorylation. May also regulate rapid eye movement (REM) sleep in the pedunculopontine tegmental (PPT). Phosphorylates APOBEC3G and AICDA. Isoform 2 phosphorylates and activates ABL1 in sperm flagellum to promote spermatozoa capacitation.
Tissue specificity	Isoform 1 is ubiquitous. Isoform 2 is sperm-specific and is enriched in pachytene spermatocytes but is not detected in round spermatids.
Sequence similarities	Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. cAMP subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 protein kinase domain.
Post-translational modifications	Asn-3 is partially deaminated to Asp giving rise to 2 major isoelectric variants, called CB and CA respectively. Autophosphorylated. Phosphorylation is enhanced by vitamin K(2). Phosphorylated on threonine and serine residues. Phosphorylation on Thr-198 is required for full activity. Phosphorylated at Tyr-331 by activated receptor tyrosine kinases EGFR and PDGFR; this increases catalytic efficienncy.
Cellular localization	Cytoplasm. Cell membrane. Nucleus. Mitochondrion. Translocates into the nucleus (monomeric catalytic subunit). The inactive holoenzyme is found in the cytoplasm. Distributed throughout the cytoplasm in meiotically incompetent oocytes. Associated to mitochondrion as meiotic competence is acquired. Aggregates around the germinal vesicles (GV) at the immature GV stage oocytes and Cell projection, cilium, flagellum. Expressed in the midpiece region of the sperm flagellum.

Images



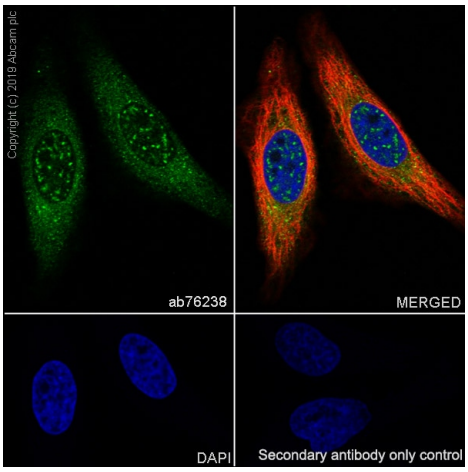
Immunoprecipitation - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

ab76238 (purified) at 1/40 dilution (2 µg) immunoprecipitating cAMP Protein Kinase Catalytic subunit in MCF-7 whole cell lysate.
Lane 1 (input): MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10 µg
Lane 2 (+): ab76238 & MCF-7 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab76238 in MCF-7 whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238)



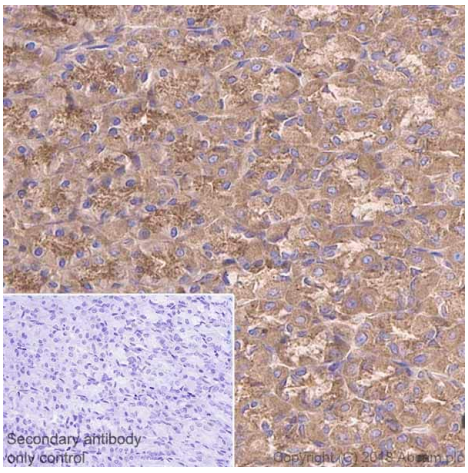
Flow Cytometry (Intracellular) - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Intracellular Flow Cytometry analysis of MCF-7 (Human breast adenocarcinoma epithelial cell) cells labeling cAMP Protein Kinase Catalytic subunit with purified ab76238 at 1/80 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238)



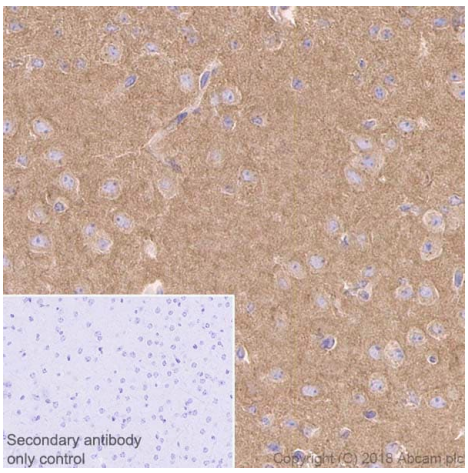
Immunocytochemistry/ Immunofluorescence - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling cAMP Protein Kinase Catalytic subunit with purified ab76238 at 1:100 dilution (8.0 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (anti camp protein kinase catalytic subunit antibody ep2102y immunocytochemistry hela human)



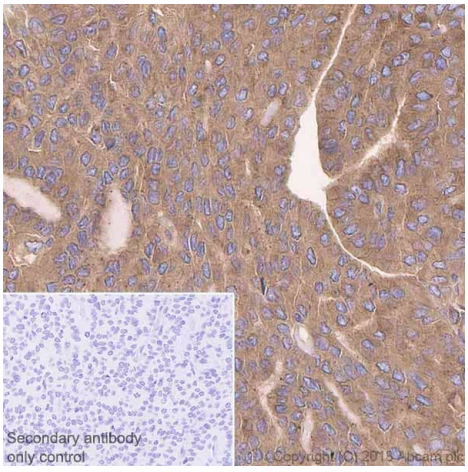
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat stomach tissue sections labeling cAMP Protein Kinase Catalytic subunit with purified ab76238 at 1/750 dilution. Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238)



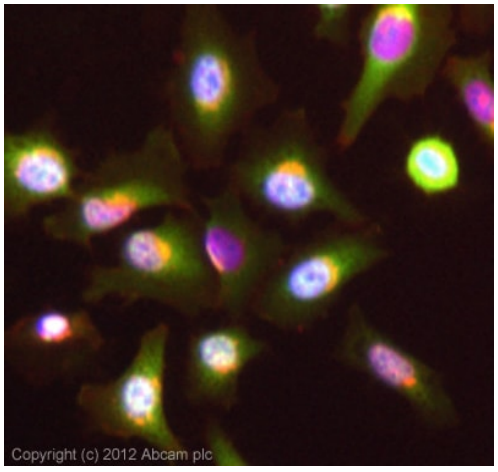
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebrum tissue sections labeling cAMP Protein Kinase Catalytic subunit with purified ab76238 at 1/750 dilution. Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

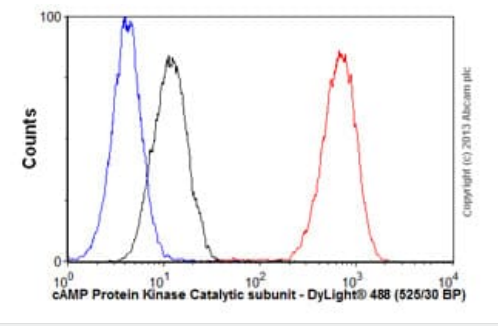
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling cAMP Protein Kinase Catalytic subunit with purified ab76238 at 1/750 dilution. Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238)



Immunocytochemistry/ Immunofluorescence - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

ICC/IF image of ab76238 (unpurified) stained HeLa cells. The cells were 4% formaldehyde (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab76238, neat) overnight at +4°C. The secondary antibody (green) was ab96899 Dylight 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

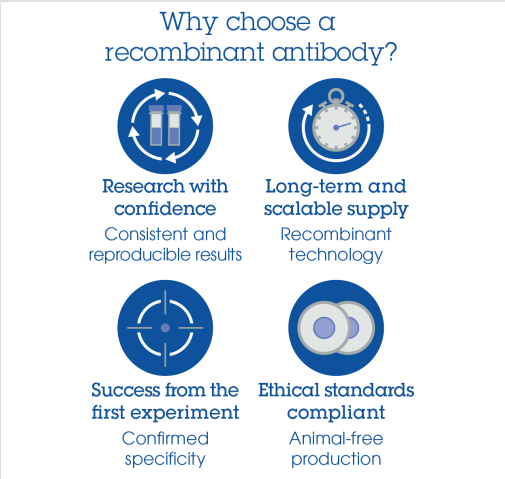
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238).



Flow Cytometry (Intracellular) - Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Overlay histogram showing HeLa cells stained with ab76238 (unpurified) (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76238, 1/100 dilution) for 30 min at 22^{O}C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22^{O}C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76238).



Anti-cAMP Protein Kinase Catalytic subunit antibody [EP2102Y] - BSA and Azide free (ab235385)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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