abcam

ab220214

Anti-CDK5 antibody [EP715Y] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP715Y
Purification technique	Affinity purification Protein A
Concentration	0.984 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Chicken
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Predicted

Species	Chicken
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Chicken
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Chicken
Dilution info	-
Notes	-

Target data

See full target information CDK5

Function	Proline-directed serine/threonine-protein kinase essential for neuronal cell cycle arrest and differentiation and may be involved in apoptotic cell death in neuronal

diseases by triggering abortive cell cycle re-entry. Interacts with D1 and D3-type G1 cyclins. Phosphorylates SRC, NOS3, VIM/vimentin, p35/CDK5R1, MEF2A, SIPA1L1, SH3GLB1, PXN, PAK1, MCAM/MUC18, SEPT5, SYN1, DNM1, AMPH, SYNJ1, CDK16, RAC1, RHOA, CDC42, TONEBP/NFAT5, MAPT/TAU, MAP1B, histone H1, p53/TP53, HDAC1, APEX1, PTK2/FAK1, huntingtin/HTT, ATM, MAP2, NEFH and NEFM. Regulates several neuronal development and physiological processes including neuronal survival, migration and differentiation, axonal and neurite growth, synaptogenesis, oligodendrocyte differentiation, synaptic plasticity and neurotransmission, by phosphorylating key proteins. Negatively regulates the CACNA1B/CAV2.2 -mediated Ca(2+) release probability at hippocampal neuronal soma and synaptic terminals (By similarity). Activated by interaction with CDK5R1 (p35) and CDK5R2 (p39), especially in postmitotic neurons, and promotes CDK5R1 (p35) expression in an autostimulation loop. Phosphorylates many downstream substrates such as Rho and Ras family small GTPases (e.g. PAK1, RAC1, RHOA, CDC42) or microtubule-binding proteins (e.g. MAPT/TAU, MAP2, MAP1B), and modulates actin dynamics to regulate neurite growth and/or spine morphogenesis. Also phosphorylates exocytosis associated proteins such as MCAM/MUC18, SEPT5, SYN1, and CDK16/PCTAIRE1 as well as endocytosis associated proteins such as DNM1, AMPH and SYNJ1 at synaptic terminals. In the mature central nervous system (CNS), regulates neurotransmitter movements by phosphorylating substrates associated with neurotransmitter release and synapse plasticity; synaptic vesicle exocytosis, vesicles fusion with the presynaptic membrane, and endocytosis. Promotes cell survival by activating anti-apoptotic proteins BCL2 and STAT3, and negatively regulating of JNK3/MAPK10 activity. Phosphorylation of p53/TP53 in response to genotoxic and oxidative stresses enhances its stabilization by preventing ubiquitin ligase-mediated proteasomal degradation, and induces transactivation of p53/TP53 target genes, thus regulating apoptosis. Phosphorylation of p35/CDK5R1 enhances its stabilization by preventing calpain-mediated proteolysis producing p25/CDK5R1 and avoiding ubiquitin ligase-mediated proteasomal degradation. During aberrant cell-cycle activity and DNA damage, p25/CDK5 activity elicits cell-cycle activity and double-strand DNA breaks that precedes neuronal death by deregulating HDAC1. DNA damage triggered phosphorylation of huntingtin/HTT in nuclei of neurons protects neurons against polyglutamine expansion as well as DNA damage mediated toxicity. Phosphorylation of PXN reduces its interaction with PTK2/FAK1 in matrix-cell focal adhesions (MCFA) during oligodendrocytes (OLs) differentiation. Negative regulator of Wnt/beta-catenin signaling pathway. Activator of the GAIT (IFN-gamma-activated inhibitor of translation) pathway, which suppresses expression of a post-transcriptional regulon of proinflammatory genes in myeloid cells; phosphorylates the linker domain of glutamyl-prolyl tRNA synthetase (EPRS) in a IFN-gamma-dependent manner, the initial event in assembly of the GAIT complex. Phosphorylation of SH3GLB1 is required for autophagy induction in starved neurons. Phosphorylation of TONEBP/NFAT5 in response to osmotic stress mediates its rapid nuclear localization. MEF2 is inactivated by phosphorylation in nucleus in response to neurotoxin, thus leading to neuronal apoptosis. APEX1 AP-endodeoxyribonuclease is repressed by phosphorylation, resulting in accumulation of DNA damage and contributing to neuronal death. NOS3 phosphorylation down regulates NOS3-derived nitrite (NO) levels. SRC phosphorylation mediates its ubiquitin-dependent degradation and thus leads to cytoskeletal reorganization. May regulate endothelial cell migration and angiogenesis via the modulation of lamellipodia formation. Involved in dendritic spine morphogenesis by mediating the EFNA1-EPHA4 signaling. The complex p35/CDK5 participates in the regulation of the circadian clock by modulating the function of CLOCK protein: phosphorylates CLOCK at 'Thr-451' and 'Thr-461' and

regulates the transcriptional activity of the CLOCK-BMAL1 heterodimer in association with altered stability and subcellular distribution.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab220214 is the carrier-free version of ab40773.

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.

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

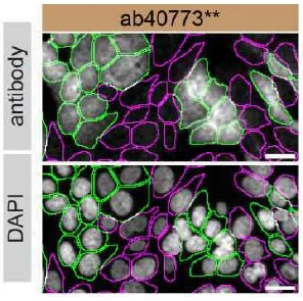
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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7 product images



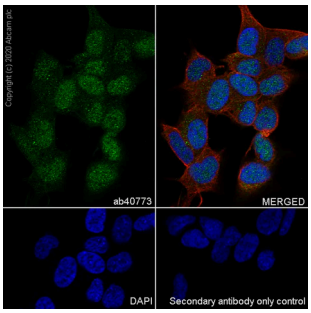
Immunocytochemistry/ Immunofluorescence - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

CDK5 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CDK5 antibody

This data was developed using ab40773, the same antibody clone in a different buffer formulation. ab40773 was shown to react with CDK5 in wild-type HEK293T cells in immunocytochemistry with loss of signal observed in CDK5 knockout cell line ab266089. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab40773 at 1/250 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and

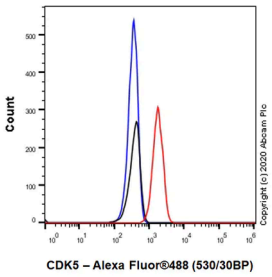
YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunocytochemistry/ Immunofluorescence - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

CDK5 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CDK5 antibody

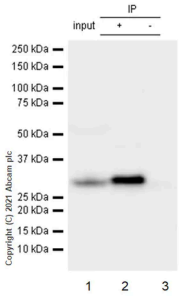
This data was developed using ab40773, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells labeling CDK5 with Purified ab40773 at 1/50 dilution (4.6 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized 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.



Flow Cytometry (Intracellular) - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

CDK5 Flow Cytometry (Intracellular) staining using rabbit Anti-CDK5 antibody

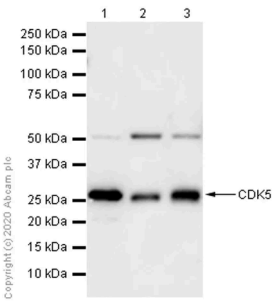
This data was developed using ab220214, the same antibody clone in a different buffer formulation.
Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling CDK5 with Purified ab220214 at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and 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). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunoprecipitation - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

This data was developed using ab220214, the same antibody clone in a different buffer formulation.
Purified ab220214 at 1:20 dilution (1µg) immunoprecipitating CDK5 in SH-SY5Y whole cell lysate.
Lane 1 (input): SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate 10µg.
Lane 2 (+): ab220214 + SH-SY5Y whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40773 in SH-SY5Y whole cell lysate.
VeriBlot for IP Detection Reagent (HRP)™(ab131366) (1:1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 33 kDa
All lanes:
Immunoprecipitation - Anti-CDK5 antibody [EP715Y] (ab40773)

Predicted band size: 33 kDa



Western blot - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

CDK5 Western blot staining using rabbit Anti-CDK5 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-CDK5 antibody [EP715Y] (ab40773) at 1/2000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 20 µg

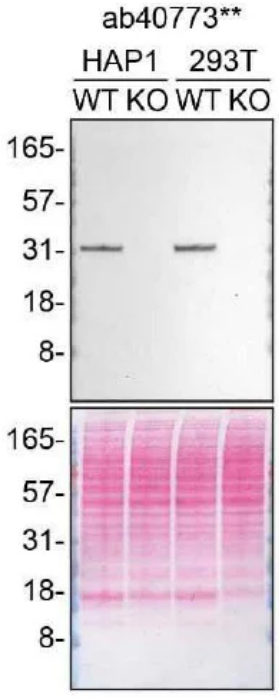
Lane 3:
PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 33 kDa

Observed band size: 33 kDa



Western blot - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

CDK5 Western blot staining using rabbit Anti-CDK5 antibody

This data was developed using ab40773, the same antibody clone in a different buffer formulation. ab40773 was shown to react with CDK5 in wild-type HEK293T cells in Western blot with loss of signal observed in a CDK5 knockout cell line. Wild-type HEK293T and CDK5 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab40773 overnight at 4 °C at a 1/2000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-CDK5 antibody [EP715Y] (ab40773) at 1/2000 dilution

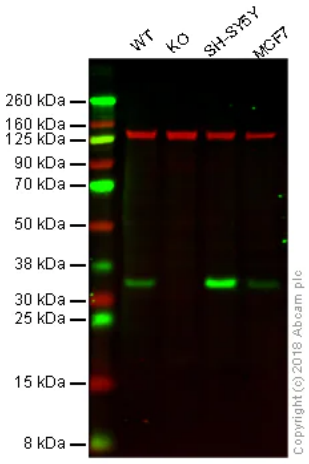
Lane 1:
Wild-type HAP1 lysate at 30 µg

Lane 2:
CDK5 knock-out HAP1 lysate at 30 µg

Lane 3:
Wild-type HEK293T lysate at 30 µg

Lane 4:
CDK5 knock-out HEK293T lysate at 30 µg

Observed band size: 33 kDa



Western blot - Anti-CDK5 antibody [EP715Y] - BSA and Azide free (ab220214)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40773).
Lanes 1 - 4: Merged signal (red and green). Green - ab40773 observed at 33 kDa. Red - loading control, ab130007, observed at 130 kDa.

ab40773 was shown to specifically react with Cdk5 in wild-type HAP1 cells as signal was lost in CDK5 knockout cells. Wild-type and CDK5 knockout samples were subjected to SDS-PAGE. ab40773 and ab130007 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-CDK5 antibody [EP715Y] (ab40773) at 1/2000 dilution

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
CDK5 knockout HAP1 whole cell lysate at 20 µg

Lane 3:
SHSY5Y whole cell lysate at 20 µg

Lane 4:
MCF7 whole cell lysate at 20 µg

Predicted band size: 33 kDa

Observed band size: 33 kDa

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