# abcam

# Product datasheet

# Anti-CDK5 antibody [EP715Y] - BSA and Azide free ab220214



Recombinant

RabMAb

7 References 6 Images

#### Overview

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

**Description** Rabbit monoclonal [EP715Y] to CDK5 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IP, ICC/IF

**Species reactivity** Reacts with: Mouse, Rat, Human

Predicted to work with: Chicken

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa whole cell lysate, NIH3T3 whole cell lysate, PC-12 whole cell lysate IP: ab220214 +

SH-SY5Y whole cell lysate. ICC/IF: SH-SY5Y (Human neuroblastoma epithelial cell)

**General notes** ab220214 is the carrier-free version of <u>ab40773</u>.

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.

Our <u>carrier-free</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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

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- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

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

# **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

ClonalityMonoclonalClone numberEP715Y

**Isotype** IgG

## **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab220214 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
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

# **Target**

**Function** Probably involved in the control of the cell cycle. Interacts with D1 and D3-type G1 cyclins. Can

phosphorylate histone H1, tau, MAP2 and NF-H and NF-M. Also interacts with p35 which

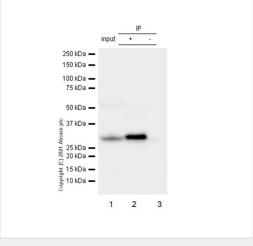
activates the kinase.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX

subfamily.

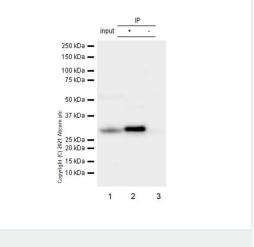
Contains 1 protein kinase domain.

#### **Images**

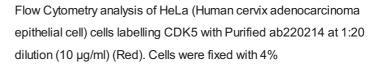


Immunoprecipitation - Anti-CDK5 antibody [EP715Y]

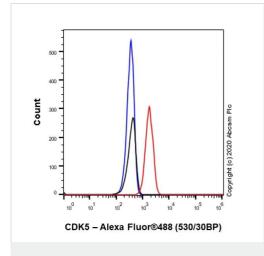
- BSA and Azide free (ab220214)



This data was developed using ab220214, the same antibody clone in a different buffer formulation.



Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (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).



Flow Cytometry (Intracellular) - 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.

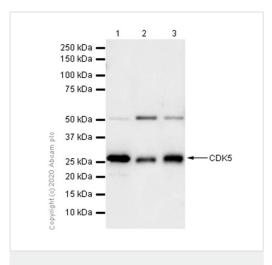
Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of ab40773 in SH-SY5Y whole cell lysate.

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



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

**All lanes :** Anti-CDK5 antibody [EP715Y] (<u>ab40773</u>) at 1/2000 dilution (Purified)

**Lane 1**: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

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

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

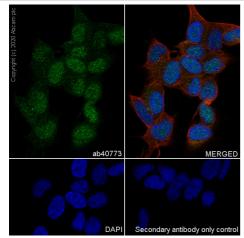
Lysates/proteins at 20 µg per lane.

# Secondary

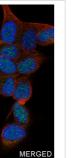
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

**Predicted band size:** 33 kDa **Observed band size:** 33 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



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



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

(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

This data was developed using ab40773, the same antibody clone

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 lgG

in a different buffer formulation.

Lane 1: Wild-type HAP1 whole cell lysate

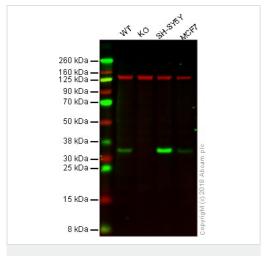
as the secondary antibody only control.

Lane 2: CDK5 knockout HAP1 whole cell lysate

Lane 3: SHSY5Y whole cell lysate Lane 4: MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 33 kDa



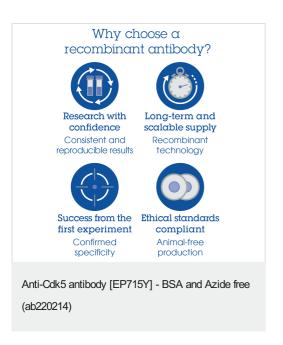
Western blot - Anti-Cdk5 antibody [EP715Y] - BSA and Azide free (ab220214)

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.



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

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