abcam

Product datasheet

Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free ab219723

Recombinant

RabMAb

8 Images

Overview

Product name Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free

Description Rabbit monoclonal [EP435Y-129R] to CDKN2A/p16INK4A +CDKN2B/p15INK4B - BSA and

Azide free

Host species Rabbit

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

Unsuitable for: IHC-P

Species reactivity Reacts with: Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK-293, HeLa ICC/IF: HeLa cells Flow Cyt (intra): HEK-293 cells. IP: HEK-293

General notes ab219723 is the carrier-free version of **ab81278**.

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 <u>conjugation kits</u> 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**.

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Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EP435Y-129R

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab219723 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
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 17 kDa.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for IHC-P.

Target

Function Acts as a negative regulator of the proliferation of normal cells by interacting strongly with CDK4

and CDK6. This inhibits their ability to interact with cyclins D and to phosphorylate the

retinoblastoma protein.

Tissue specificity Widely expressed but not detected in brain or skeletal muscle. Isoform 3 is pancreas-specific.

Involvement in diseaseThe association between cutaneous and uveal melanomas in some families suggests that

mutations in CDKN2A may account for a proportion of uveal melanomas. However, CDKN2A

mutations are rarely found in uveal melanoma patients.

Melanoma, cutaneous malignant 2

Familial atypical multiple mole melanoma-pancreatic carcinoma syndrome

Melanoma-astrocytoma syndrome

Sequence similaritiesBelongs to the CDKN2 cyclin-dependent kinase inhibitor family.

Contains 4 ANK repeats.

Post-translational

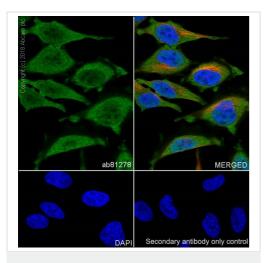
modifications

Phosphorylation seems to increase interaction with CDK4.

Cellular localization

Cytoplasm. Nucleus.

Images

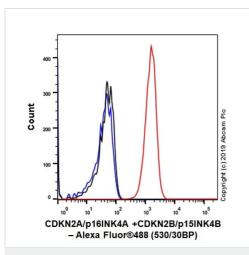


Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)

Immunofluorescent analysis of 4% Paraformaldehyde fixed 0.1% Triton X-100 permeabilised HeLa (human cervix adenocarcinoma epithelial cell) labelling CDKN2A/p16INK4a with <u>ab81278</u> at 1/250 dilution, followed by AlexaFluor[®] 488 Goat anti-Rabbit secondary antibody (<u>ab150077</u>) at 1/1000 dilution (green). Confocal image showing cytoplasmic and nuclear staining in HeLa is observed. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (<u>ab195889</u>) was used to counterstain alpha Tubulin at 1/200 dilution (red). The Nuclear counterstain was DAPI (blue).

Secondary antibody only control: Secondary antibody is AlexaFluor[®] 488 Goat anti-Rabbit secondary antibody (<u>ab150077</u>) at 1/1000 dilution (green).

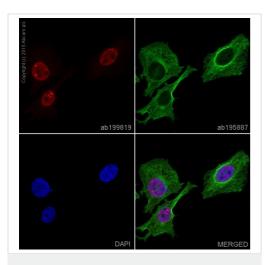
This data was developed using <u>ab81278</u>, the same antibody clone in a different buffer formulation.



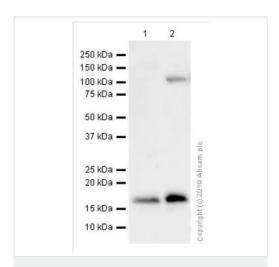
Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)

Flow cytometric analysis of 4% paraformaldehyde fixed and 90% methanol permeabilised HEK-293 (Human embryonic kidney epithelial cell) with <u>ab81278</u> at 1/20 dilution (red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

This data was developed using <u>ab81278</u>, the same antibody clone in a different buffer formulation.



Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)



Western blot - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)

Clone EP435Y-129R (ab219723) has been successfully conjugated by Abcam. This image was generated using Anti-CDKN2A/p16INK4A +CDKN2B/p15INK4B antibody [EP435Y-129R] (Alexa Fluor[®] 647). Please refer to **ab199819** for protocol details.

<u>ab199819</u> staining CDKN2A/p16INK4a in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab199819</u> at 1/200 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 488), at $2\mu g/ml$ (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

All lanes : Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723) at 1/10000 dilution

Lane 1 : HEK-293 cell lysate
Lane 2 : HeLa cell lysate

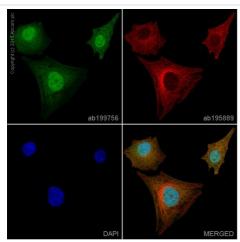
Lysates/proteins at 1/20 dilution per lane.

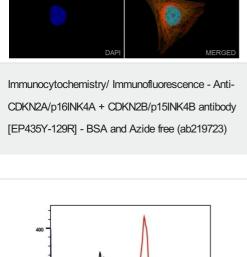
Secondary

All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/20000 dilution

Predicted band size: 17 kDa

Exposure time: 3 seconds





Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)

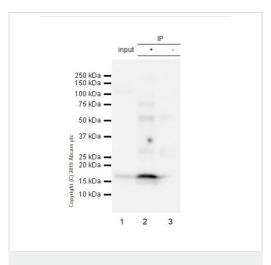
CDKN2A/p16INK4a - Alexa Fluor® 488 (530/30 BP)

Clone EP435Y-129R (ab219723) has been successfully conjugated by Abcam. This image was generated using Anti-CDKN2A/p16lNK4a antibody [EP435Y-129R] (Alexa Fluor® 488). Please refer to <u>ab199756</u> for protocol details.

ab199756 staining CDKN2A/p16INK4a in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab199756** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 594), at 2μg/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HEK-293 (Human embryonic kidney epithelial cell) cells labelling CDKN2A with ab219723 at 1/100 dilution (1µg)/ Red compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Immunoprecipitation - Anti-CDKN2A/p16INK4A + CDKN2B/p15INK4B antibody [EP435Y-129R] - BSA and Azide free (ab219723)

CDKN2A/p16INK4a was immunoprecipitated from 0.35 mg HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg with ab219723 at 1:50 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab219723 1:1000 dilution (2 µg/ml). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

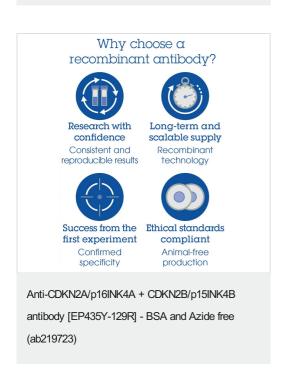
Lane 1: HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg

Lane 2: ab219723 IP in HEK-293 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab219723 in HEK-293 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds



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