

Product datasheet

Anti-CDKN2A/p19ARF antibody [5-C3] ab26696

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Overview

Product name	Anti-CDKN2A/p19ARF antibody [5-C3]
Description	Rat monoclonal [5-C3] to CDKN2A/p19ARF
Host species	Rat
Tested applications	Suitable for: WB, IP, ICC/IF, IHC-FoFr
Species reactivity	Reacts with: Mouse Does not react with: Hamster, Human
Immunogen	Synthetic peptide corresponding to Mouse CDKN2A/p19ARF conjugated to keyhole limpet haemocyanin.
Positive control	ICC/IF: MEF1 cells WB: MEF1 cell lysate, NIH3T3 cells
General notes	<p>The 5-C3 monoclonal has been knockout validated in WB and ICC/IF using wild type and knockout mouse samples. The data are shown in the images section.</p> <p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.50 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified

Clonality	Monoclonal
Clone number	5-C3
Myeloma	Y3/Ag1.2.3
Isotype	IgG2b

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab26696 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 a concentration of 1 µg/ml. Detects a band of approximately 19 kDa (predicted molecular weight: 17 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		1/1000.
IHC-FoFr		Use at an assay dependent concentration.

Target

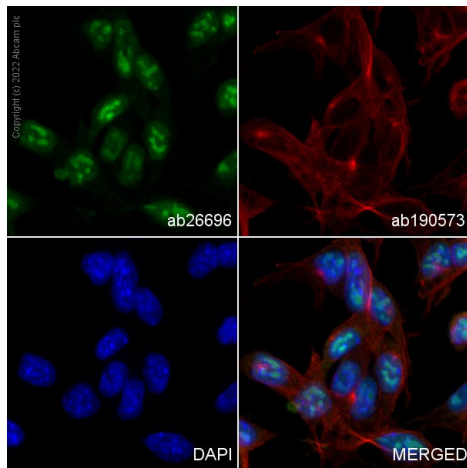
Relevance

The gene for CDK2NA generates several transcripts/proteins which differ from each other in their first exons. Three of these transcripts are generated by alternative splicing (isoform 1 a.k.a p16INK4A, isoform 2 and isoform 3 a.k.a p12), two of which are known to function as inhibitors of CDK4 kinase. One other transcript that is generated from this gene contains an alternate reading frame (ARF), with the first exon located 20kb upstream of the remainder of the gene (isoform 4 a.k.a. p14ARF, p19ARF, ARF). In spite of the structural and some functional differences, all the proteins encoded by the CDKN2A gene are involved in cell cycle G1 control.

Cellular localization

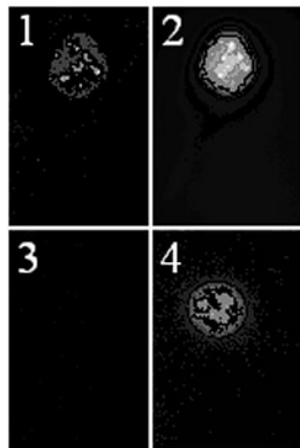
Nuclear

Images



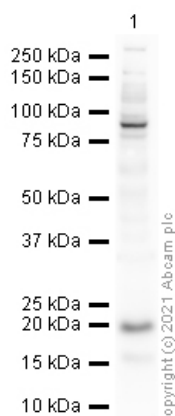
Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696)

ab26696 staining CDKN2A/p19ARF in MEF1 cells. The cells were fixed with 4% PFA (10 mins), 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 ab26696 at 5µg/ml and **ab190573**, Rabbit monoclonal to alpha Tubulin (Alexa Fluor® 647), at 2µg/ml (shown in red). The secondary antibody (shown in green) was **ab150165**, Alexa Fluor® 488 Goat anti-Rat IgG (H+L) used at a 1/1000 dilution for 1h at room temperature. Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696)

Immunofluorescent detection of p19ARF. MEF cells (1 and 2) or NIH3T3 cells with arf gene deleted (3 and 4) were stained with anti-p19ARF 5C3 followed by a fluorescent-labelled anti-rat IgG (1 and 3) and Hoechst dye to visualise nuclei (2 and 4).



Western blot - Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696)

Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696) at 2 µg/ml + MEF-1 whole cell lysate at 20 µg

Secondary

Goat polyclonal to Rat IgG - H&L - Pre-Adsorbed (HRP) at 1/10000 dilution

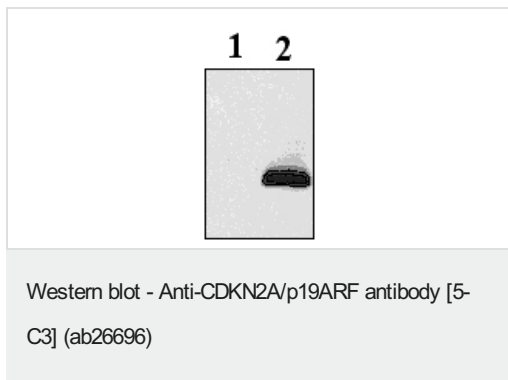
Predicted band size: 17 kDa

Observed band size: 19,85 kDa

Exposure time: 1 minute

Blocking buffer: 2% BSA

Gel type: MES



All lanes : Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696)

Lane 1 : Total cell lysate from NIH3T3 cells with Arf gene deleted

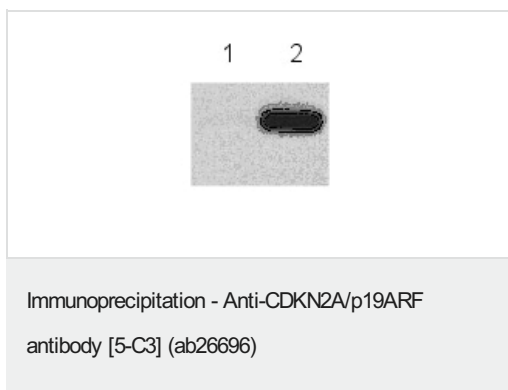
Lane 2 : MEF total cell lysate

Secondary

All lanes : anti-rat IgG-HRP

Predicted band size: 17 kDa

Observed band size: 19 kDa



Immunoprecipitation using anti-p19ARF 5C3. NIH3T3 cells expressing HA-p19ARF were incubated with a control rat IgG (lane 1) or anti-p19ARF 5C3 (2). Precipitated proteins were analysed by Western blot using anti-HA antibody.

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