

## Product datasheet

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

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#### Overview

<b>Product name</b>	Anti-CDKN2A/p19ARF antibody [5-C3]
<b>Description</b>	Rat monoclonal [5-C3] to CDKN2A/p19ARF
<b>Host species</b>	Rat
<b>Tested applications</b>	<b>Suitable for:</b> WB, IP, ICC/IF, IHC-FoFr
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse <b>Does not react with:</b> Hamster, Human
<b>Immunogen</b>	Synthetic peptide corresponding to Mouse CDKN2A/p19ARF conjugated to keyhole limpet haemocyanin.
<b>Positive control</b>	ICC/IF: MEF1 cells WB: MEF1 cell lysate, NIH3T3 cells
<b>General notes</b>	<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 <a href="mailto:orders@abcam.com">orders@abcam.com</a>.</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&amp;As</p>

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.50 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
<b>Purity</b>	Protein G purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	5-C3
<b>Myeloma</b>	Y3/Ag1.2.3
<b>Isotype</b>	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.

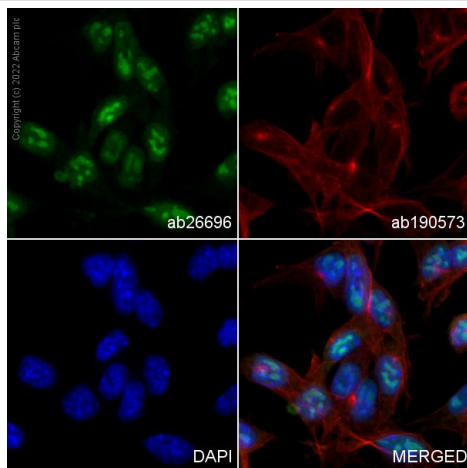
Application	Abreviews	Notes
<b>WB</b>		Use a concentration of 1 µg/ml. Detects a band of approximately 19 kDa (predicted molecular weight: 17 kDa).
<b>IP</b>		Use at an assay dependent concentration.
<b>ICC/IF</b>		1/1000.
<b>IHC-FoFr</b>		Use at an assay dependent concentration.

## 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.

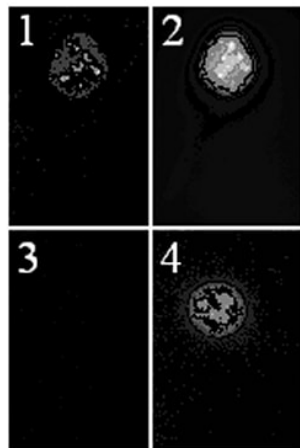
**Cellular localization** Nuclear

## Images



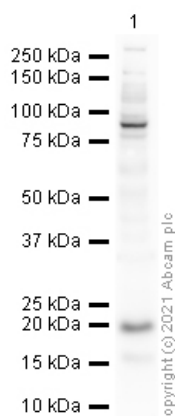
Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p19ARF antibody [5-C3] (ab26696)

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

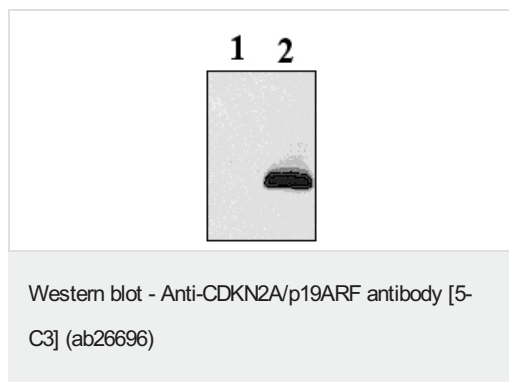
**Predicted band size:** 17 kDa

**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

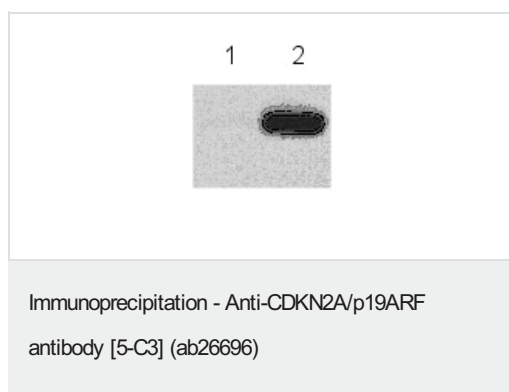
**Lane 2 :** MEF total cell lysate

#### 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.

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

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