

Product Datasheet

p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free NB200-174

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NB200-174

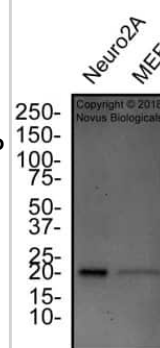
p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	5-C3-1
Preservative	0.02% Sodium Azide
Isotype	IgG2b
Purity	Protein A purified
Buffer	PBS
Target Molecular Weight	19 kDa
Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rat p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free (NB200-174) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-p19ARF/CDKN2A Antibody: Cited in 24 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rat
Gene ID	12578
Gene Symbol	Cdkn2a
Species	Human, Mouse, Golden Syrian Hamster (Negative)
Reactivity Notes	Human reactivity reported in scientific literature (PMID: 27177758). The antibody does not react with Golden Syrian hamster. Armenian hamster has not been tested.
Immunogen	A synthetic peptide containing amino acids 54-75 of the murine p19ARF/CDKN2A. [UniProt# Q64364]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, CyTOF-ready, Knockout Validated
Recommended Dilutions	Western Blot 1:500-1:1000, Flow Cytometry 1 ug/mL, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500-1:1000, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin reported in scientific literature (PMID 27177758), Immunohistochemistry-Frozen 5 ug/ml, Flow (Intracellular), CyTOF-ready, Knockout Validated
Application Notes	In Western blot a band can be seen at ~19 kDa. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

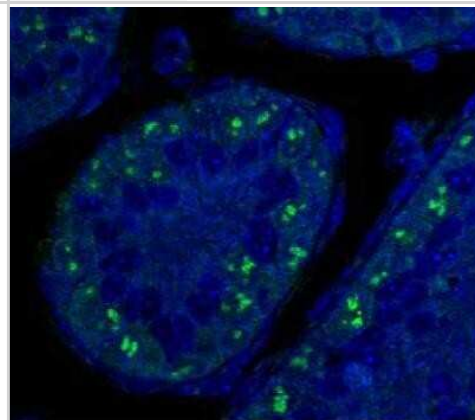


Images

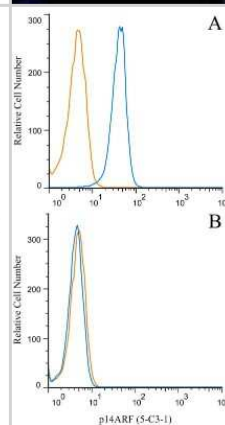
Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - Whole cell protein from mouse Neuro2A and MEF cells was separated on a 4-20% gel by SDS-PAGE, transferred to 0.2 um PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/mL anti-p19ARF in block buffer and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



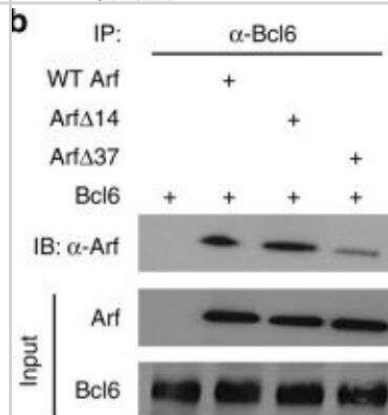
Immunohistochemistry: p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - Staining of sections from a wild type mouse testis taken at P11 were probed with NB200-174 (green). Sections were stained with DAPI to reveal the position of cell nuclei (blue).



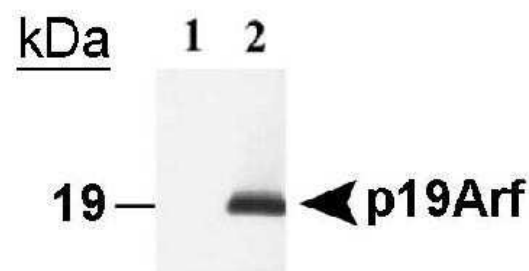
Flow (Intracellular): p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - An intracellular stain was performed on HEK293 cells with p14ARF (5-C3-1) antibody NB200-174 (blue) and a matched isotype control MAB0061 (orange) [A]. An intracellular stain was also performed on THP-1 (known homozygous deletion of gene, PMID: 19635138) cells to ensure antibody specificity [B]. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Goat Anti-Rat F(ab)2 IgG (H+L) APC-conjugated Antibody (F0113, R&D Systems). *Note: This antibody recognizes both human (p14ARF) and mouse (p19ARF) versions of the protein.



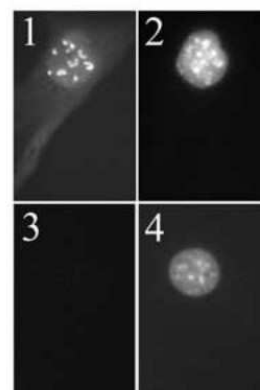
Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - Aberrantly upregulated p19ARF/CDKN2A antagonizes TFH differentiation and survival. p19ARF/CDKN2A and Bcl6 interaction capacity. WT or mutant p19ARF/CDKN2A was co-transfected with WT Bcl6 expression vector into 293T cells. Cell lysates were immunoprecipitated with anti-Bcl6 and then immunoblotted with anti-p19ARF/CDKN2A. Data are representative of two experiments. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-018-07853-z>) licensed under a CC-BY license.



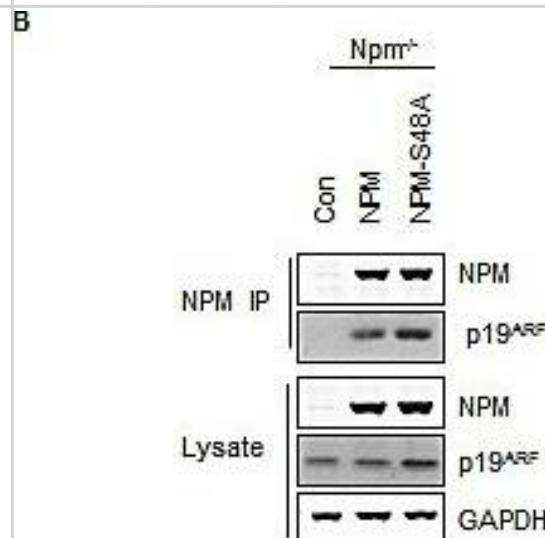
Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - Analysis of endogenous p19Arf. Total cell lysates (25 ug) from NIH3T3 cells, which have deleted the Arf gene (Lanes 1) and from wild type mouse embryo fibroblasts (MEFs) at passage 6, which express p19Arf (Lanes 2), were resolved by SDS-PAGE.



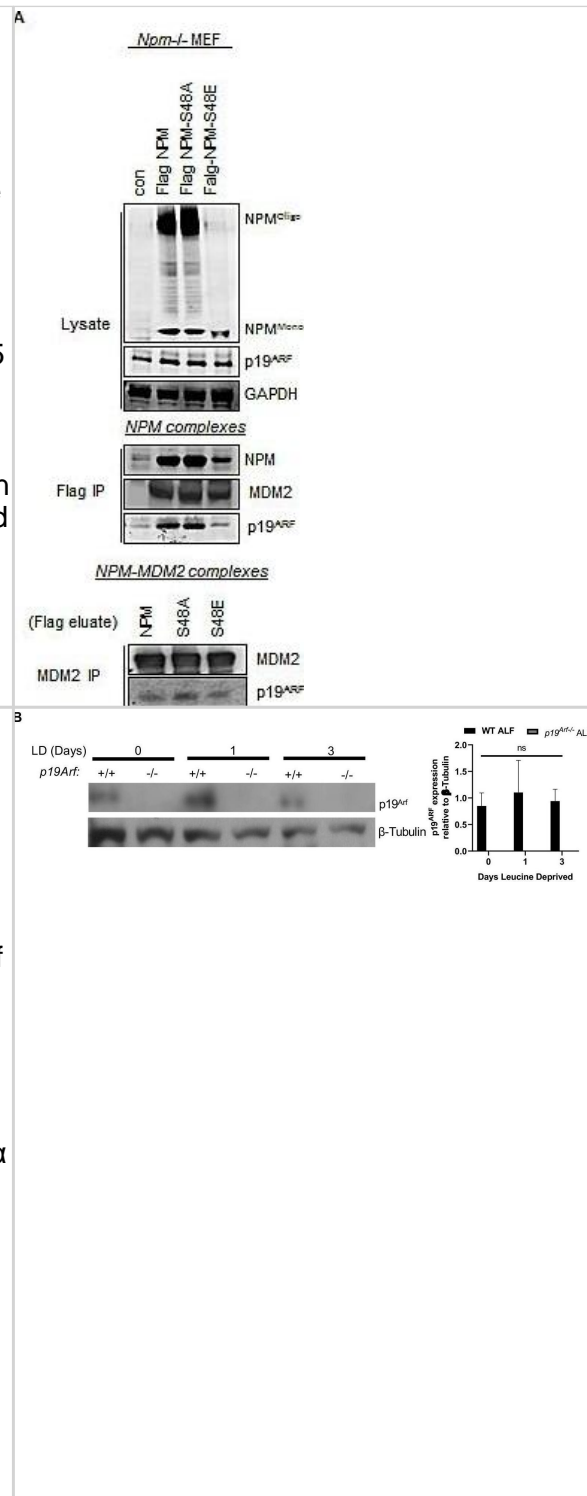
Immunocytochemistry/ Immunofluorescence: p19ARF/CDKN2A Antibody (5-C3-1) [NB200-174] - Detection of p19Arf. Wild type mouse embryo fibroblasts (MEF) at passage 5 (panels 1, 2) and NIH3T3 cells, which have deleted the Arf gene (panels 3, 4) were probed with NB200-17 and nuclear stain (overlay in panels 2 and 4).



Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free [NB200-174] - Phosphorylation of NPM-Ser48 regulates the localization of NPM & p19A(A) Npm^{-/-}, p53^{-/-} double null MEF were infected with pBabe retrovirus expressing FLAG-tagged-myr-AKT1 in combination with NPM-WT or NPM-S48A as indicated. Cells were fixed & stained with DAPI & anti-NPM (left) or anti-phospho-S48-NPM (pS48-NPM). (B) NPM immunoprecipitates & whole cell lysates from Npm^{-/-};p53^{-/-} MEFs expressing human NPM or NPM-S48A were probed with the indicated antibodies. (C) Graph, quantification of p19ARF staining intensity in confocal images using ImageJ. (D) Npm^{-/-}, p53^{-/-} double null MEF were infected with pBABE retrovirus expressing FLAG-tagged-myr-AKT1 in combination with NPM-WT, NPM-S48A or S48E as indicated. Cells were fixed & stained with DAPI, anti-NPM & anti p19ARF. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25071014>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

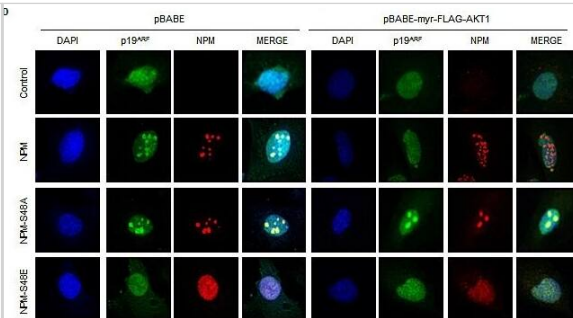


Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free [NB200-174] - Inhibition of AKT promotes enhanced MDM2 activity via the increased association between NPM & p14ARF(A) Npm^{-/-}, p53^{-/-} double null MEF were infected with pBABE retrovirus empty vector & pBABE expressing FLAG-tagged-NPM-WT, NPM-S48A or S48E as indicated. Immunoprecipitation of NPM was done by pulling down with the Flag tag (middle panel) followed by elution of complexes by the Flag peptide & subsequent immunoprecipitation of endogenous MDM2 (lower panel). (B) Nuclear immunoprecipitates of MDM2 from T24 cells treated with MK-2206 (5 μ M, 24 hrs). Immunoprecipitates & lysates were blotted with the indicated antibodies. (C) T24 cells were treated with MK-2206 (5 μ M) as indicated. p14ARF was immunoprecipitated from whole cell lysates & nuclear extracts & the association with NPM & MDM2 determined by western blot. Immunoprecipitates & lysates were blotted with the indicated antibodies. (D) MDM2 & (E) p53 ubiquitination assay in H1299 cells transfected with wild type p53, HA-tagged ubiquitin & treated for 16 hrs with DMSO, MK-2206 (5 μ M) or Nutlin3A (5 μ M) as indicated. Immunoprecipitates & whole cell lysates were probed with the indicated antibodies. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25071014>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

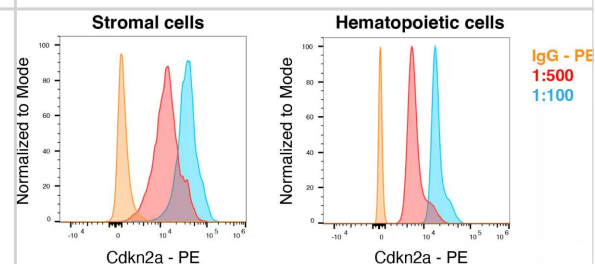


Western Blot: p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free [NB200-174] - Loss of P19Arf enhances tumor growth & primary lung fibroblasts induce p19Arf in response to leucine deprivation (LD). (A) Graph of sarcoma (SKPY) tumor volume on the indicated days. SKPY cells were injected into the flank of WT & p19Arf-null mice. Tumor volume was measured by caliper. N=7 mice/WT cohort & N=15 mice/p19Arf^{-/-} cohort. (B) Western blot for p19Arf protein expression in WT & Arf-null fibroblasts after LD for the indicated days. Densitometric quantification of p19Arf protein expression relative to β -tubulin is shown on right. (C) qPCR for p19Arf mRNA in WT or p19Arf^{-/-} fibroblasts during LD for the indicated days. (D) Representative immunofluorescence images for P19Arf subcellular localization on the indicated days after LD in WT or p19Arf^{-/-} ALFs. Quantification of nuclear p19Arf is shown in graph on right. (E) Western blot analysis of phospho-eIF2 α (P-eIF2 α) & total eIF2 α expression in WT & p19Arf-null murine ALFs during LD for the indicated days. Graph quantifies intensity of P-eIF2 α expression relative to day 0. β -Tubulin used as loading control. (F) Western blot analysis of P-eIF2 α expression in WT & p19Arf^{-/-} ALFs upon treatment with a GCN2 inhibitor (GCN2-IN-1; A-92 1 μ M in DMSO) during overnight LD. Actin used as a loading control. Quantification of P-eIF2 α expression relative to total eIF2 α . N=3; *, P<0.05; ns, not significant. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/34994382>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free [NB200-174] - Phosphorylation of NPM-Ser48 regulates the localization of NPM & p19A(A) Npm^{-/-}, p53^{-/-}-double null MEF were infected with pBabe retrovirus expressing FLAG-tagged-myr-AKT1 in combination with NPM-WT or NPM-S48A as indicated. Cells were fixed & stained with DAPI & anti-NPM (left) or anti-phospho-S48-NPM (pS48-NPM). (B) NPM immunoprecipitates & whole cell lysates from Npm^{-/-};p53^{-/-} MEFs expressing human NPM or NPM-S48A were probed with the indicated antibodies.(C) Graph, quantification of p19ARF staining intensity in confocal images using ImageJ. (D) Npm^{-/-}, p53^{-/-}-double null MEF were infected with pBabe retrovirus expressing FLAG-tagged-myr-AKT1 in combination with NPM-WT, NPM-S48A or S48E as indicated. Cells were fixed & stained with DAPI, anti-NPM & anti p19ARF. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25071014>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Flow Cytometry: p19ARF/CDKN2A Antibody (5-C3-1) - BSA Free - Conducted the antibody trial with two different dilutions of p19ARF/CDKN2A Antibody (5-C3-1) [PE] (Catalog # NB200-174PE) (1:500 and 1:100) and compared them to an IgG control which was also PE labelled. Image from a verified customer review.



Publications

Roby KC, Lieberman A, Kim BJ Et al. Loss of p19Arf promotes fibroblast survival during leucine deprivation Biol Open 2022-01-07 [PMID: 34994382]

Details:

Citation using the DyLight 488 version of this antibody.

Marloes C M Jonkhout, Tijs Vanhessche, Mónica Ferreira, Iris Verbinnen, Fabienne Withof, Gerd Van der Hoeven, Kathelijne Szekér, Zahra Azhir, Wen-Hui Lien, Aleyde Van Eynde, Mathieu Bollen Embryonic NIPP1 Depletion in Keratinocytes Triggers a Cell Cycle Arrest and Premature Senescence in Adult Mice. The Journal of investigative dermatology 2024-02-29 [PMID: 38431220]

Weidenhammer L, Liu H, Luo L et al. Inducing primary brainstem gliomas in genetically engineered mice using RCAS/TVA retroviruses and Cre/loxP recombination STAR Protocols 2023-03-01 [PMID: 36853662]

Deland, K, Starr, B F Et al. Tumor genotype dictates radiosensitization after Atm deletion in primary brainstem glioma models. J Clin Invest 2021-01-04 [PMID: 32990677] (WB, Mouse)

Maru Y, Tanaka N, Tatsumi Y et al. Kras activation in endometrial organoids drives cellular transformation and epithelial-mesenchymal transition Oncogenesis 2021-06-25 [PMID: 34172714] (WB, Mouse)

Suzuki T, Katada E, Mizuoka Y, et al. A novel all-in-one conditional knockout system uncovered an essential role of DDX1 in ribosomal RNA processing Nucleic acids research 2021-01-27 [PMID: 33503245] (WB, Mouse)

Neupane R BMI1 is a context-dependent tumor suppressor that is a barrier to dedifferentiation in non-small cell lung adenocarcinoma Thesis 2019-05-23 (IF/IHC, Mouse)

Santelli A, Sun I, Eirin A, et al. Senescent Kidney Cells in Hypertensive Patients Release Urinary Extracellular Vesicles J Am Heart Assoc 2019-08-22 [PMID: 31433703] (FLOW, Human)

Li F, Zeng Z, Xing S et al. Ezh2 programs TFH differentiation by integrating phosphorylation-dependent activation of Bcl6 and polycomb-dependent repression of p19Arf Nat Commun 2018-12-21 [PMID: 30575739] (WB, Human)

Baek KH, Ryeom S. Detection of Oncogene-Induced Senescence In Vivo. Methods Mol. Biol. 2016-11-04 [PMID: 27812880]

Moding EJ, Min HD, Castle KD et al. An extra copy of p53 suppresses development of spontaneous Kras-driven but not radiation-induced cancer JCI Insight 2016-07-07 [PMID: 27453951]

Li D, Fu J, Du M et al. Hepatocellular carcinoma repression by TNFalpha-mediated synergistic lethal effect of mitosis defect-induced senescence and cell death sensitization. Hepatology. 2016-05-14 [PMID: 27177758] (IHC-P, ICC/IF, Human, Mouse)

More publications at <http://www.novusbio.com/NB200-174>



Procedures

Western Blot protocol specific for p19ARF Antibody (NB200-174)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunoprecipitation protocol for p19ARF/CDKN2A Antibody (NB200-174)

Immunoprecipitation

1. Total cell lysates (0.25 mg) from NIH3T3 cells which have deleted the Arf gene and from NIH3T3 cells expressing an HA-tagged version of p19Arf were incubated with control rat IgG or anti-p19Arf (NB 200-174) and protein G-Sepharose.
2. Precipitated proteins were resolved by SDS-PAGE and analysed by Western blotting using an anti-HA antibody. Unprecipitated total cell lysates (25 ug, equivalent to 10% of immunoprecipitation) from Arf null NIH3T3 cells and HA-p19Arf expressing cells were also analysed by Western blotting, as input controls.





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HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
F0105B	Goat anti-Rat IgG Secondary Antibody [Phycoerythrin]
DDXCR03	Rat IgG2b Isotype Control
NB200-174H	p19ARF/CDKN2A Antibody (5-C3-1) [HRP]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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