

Product Datasheet

p14ARF/CDKN2A Antibody - BSA Free NB200-111SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB200-111SS

p14ARF/CDKN2A Antibody - BSA Free

Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

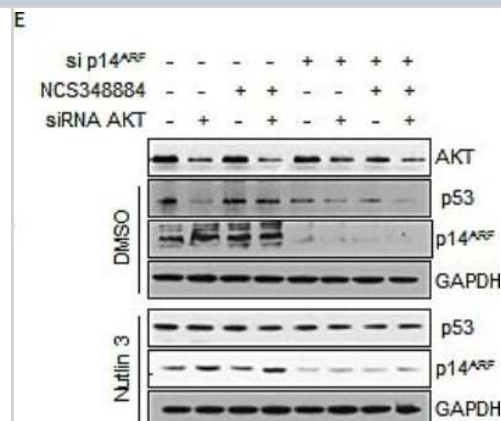
Product Description	
Description	Novus Biologicals Rabbit p14ARF/CDKN2A Antibody - BSA Free (NB200-111) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-p14ARF/CDKN2A Antibody: Cited in 18 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	1029
Gene Symbol	CDKN2A
Species	Human, Mouse
Reactivity Notes	Human and mouse reactivity reported in scientific literature.
Immunogen	A synthetic peptide made to a portion of human p14ARF (between residues 50-132). [Swiss-Prot# Q8N726]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Knockdown Validated
Recommended Dilutions	Western Blot reported in scientific literature (PMID 21636682), Flow Cytometry 1:400, Immunohistochemistry 1:10-1:500. Use reported in scientific literature (PMID 18505964), Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 25071014), Immunoprecipitation reported in scientific literature (PMID 20699639), Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen 1:10-1:500. Use reported by customer review, Knockdown Validated
Application Notes	This p14ARF antibody can be used for Western blotting, where a band is seen at ~16 kDa, representing p14ARF. Additional faint bands may be seen at ~32 and 47 kDa. In ICC/IF [PMID: 25071014], nuclear focal staining was observed in HeLa cells.

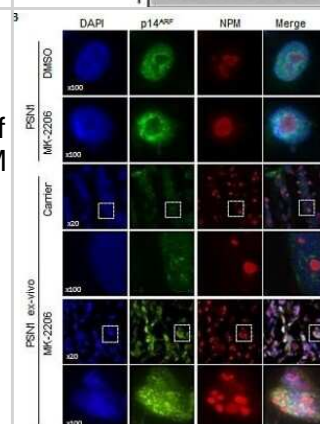


Images

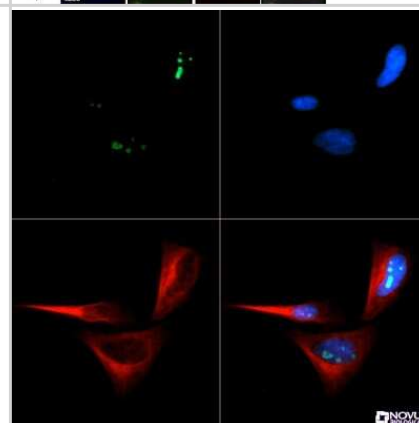
Western Blot: p14ARF/CDKN2A Antibody [NB200-111] - Inhibition of AKT decreases p53mut stability. T24 cells were transfected with non-targeting control, AKT1, or p14ARF siRNA. Cells were treated with NCS348884 (4 i1/4M), Nutlin3A (5 i1/4M) or DMSO as indicated. Whole cell lysates were probed with the indicated antibodies. Image collected and cropped by Citeab from the following publication (AKT regulates NPM dependent ARF localization and p53mut stability in tumors. *Oncotarget* (2014)) licensed under a CC-BY license.



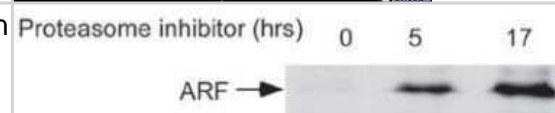
Immunohistochemistry: p14ARF/CDKN2A Antibody [NB200-111] - Inhibition of AKT modulates p53 stability in-vivo and synergizes with ionizing radiation to inhibit tumor growth(Sections of PSN1 xenografts treated with three consecutive doses of MK-2206 (60 mg/kg). Sections of PSN1 xenografts and in-vitro PSN1 cells fixed and stained with anti-NPM (red) and anti-p14ARF (green). Image collected and cropped by Citeab from the following publication (AKT regulates NPM dependent ARF localization and p53mut stability in tumors. *Oncotarget* (2014)) licensed under a CC-BY license.



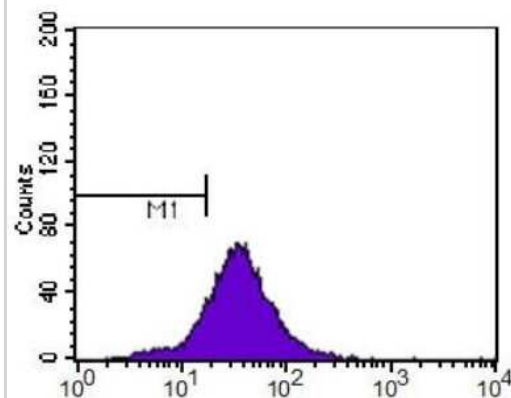
Immunocytochemistry/Immunofluorescence: p14ARF/CDKN2A Antibody [NB200-111] - p14ARF antibody was tested in HeLa cells with Dylight 488 (green). Nuclei were counterstained with DAPI (blue). Tubulin was stained with alpha tubulin (red).



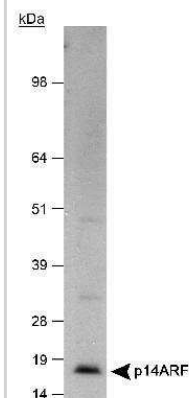
Western Blot: p14ARF/CDKN2A Antibody [NB200-111] - ARF expression levels in OCI/AML3 cells after proteasome treatment. This image was submitted via customer Review.



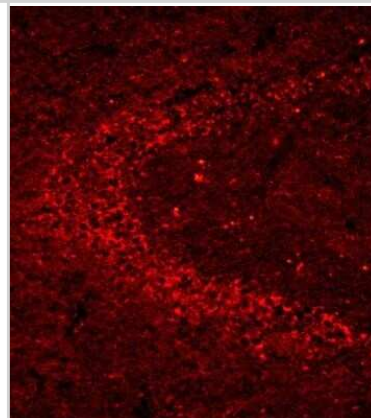
Flow Cytometry: p14ARF/CDKN2A Antibody [NB200-111] - p14ARF antibody was tested at 1:400 in HeLa cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.



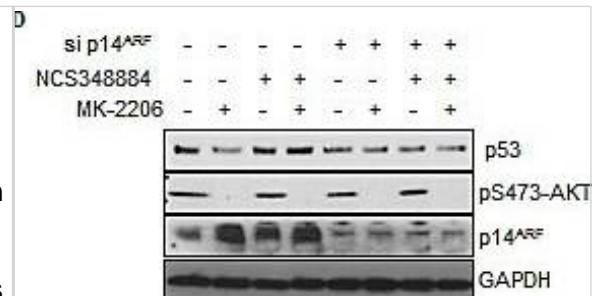
Western Blot: p14ARF/CDKN2A Antibody [NB200-111] - Analysis of HeLa Whole Cell Lysate (NB800-PC1) using p14ARF antibody (lot C) employing ECL detection method.



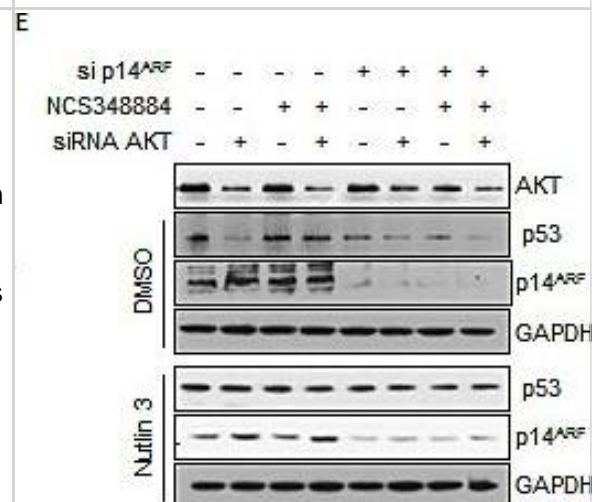
Immunohistochemistry-Frozen: p14ARF/CDKN2A Antibody [NB200-111] - Mouse brain section, hippocampal (10 um thickness). 3% PFA perfused. 1:1000 dilution. Image from verified customer review. Image using the Biotin form of this antibody.



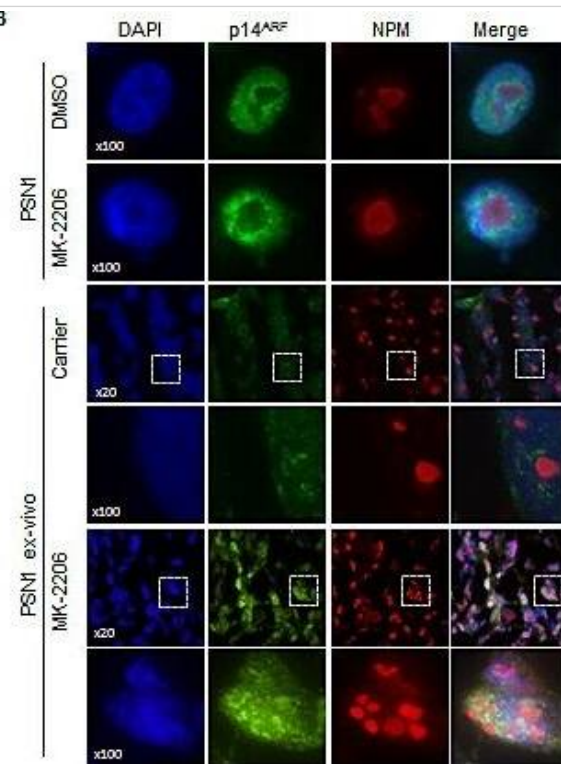
Western Blot: p14ARF/CDKN2A Antibody - BSA Free [NB200-111] - Inhibition of AKT decreases p53mut stability(A) T24 cells & PSN1 cells were treated with MK-2206 (5 μ M) or DMSO for 24 hrs. Cells were fixed & stained with DAPI & anti-mutant p53 (OP29 clone). (B) T24 cells were pre-treated with MK-2206 (5 μ M) or DMSO for 24 hrs before the addition of fresh media containing cyclohexamide (100 μ M) (CHX) in combination with MK-2206 (5 μ M) or DMSO for the times indicated. Nuclear extracts were prepared from treated cells & blotted with the indicated antibodies. Quantification is relative to initiation of CHX treatment for both conditions *, MK-2206 is 40% of DMSO control but taken as 1.0 for relative assessment. (C) H1299 cells were transfected with HA-tagged-ubiquitin & mutant p53 (R175H or R248W) as indicated. Transfected cells were treated with DMSO, MK-2206 (5 μ M) or Nutlin3A (5 μ M) for 16hrs as indicated. p53 immunoprecipitates & whole cell lysates were probed with the indicated antibodies. (D) T24 cells were transfected with non-targeting control or p14ARF siRNA & treated with DMSO, MK-2206 (5 μ M) or the NPM oligomerisation inhibitor NCS348884 (4 μ M) (Qi et al., 2008) as indicated. (E) T24 cells were transfected with non-targeting control, AKT1, or p14ARF siRNA. Cells were treated with NCS348884 (4 μ M), Nutlin3A (5 μ M) or DMSO as indicated. Whole cell lysates were probed with the indicated antibodies. (F) KPC mice derived KRASG12D p53 Floxed (p53FI), KRASG12D p53R172H ARF+/+ & KRASG12D p53R172H ARF-/- pancreatic tumor cells were treated with MK-2206 (1 μ M), Nutlin3A (5 μ M) or DMSO as indicated. 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.



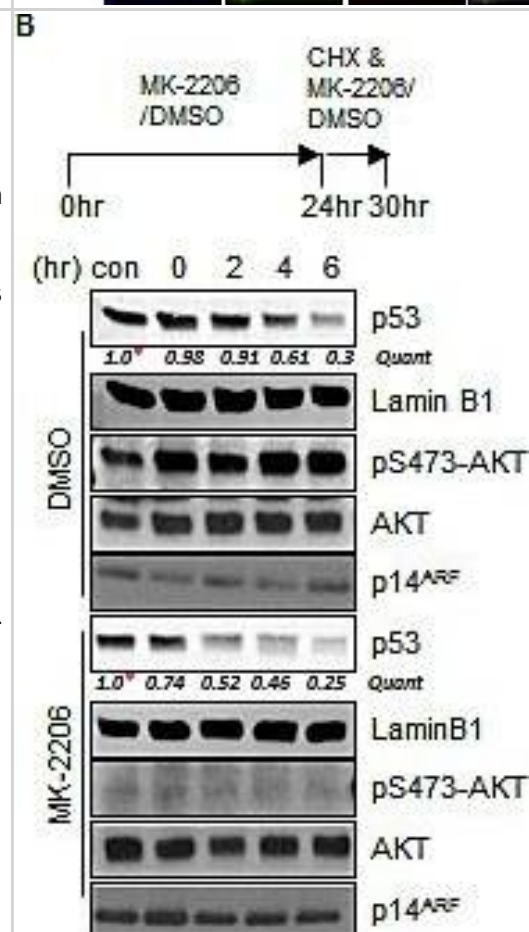
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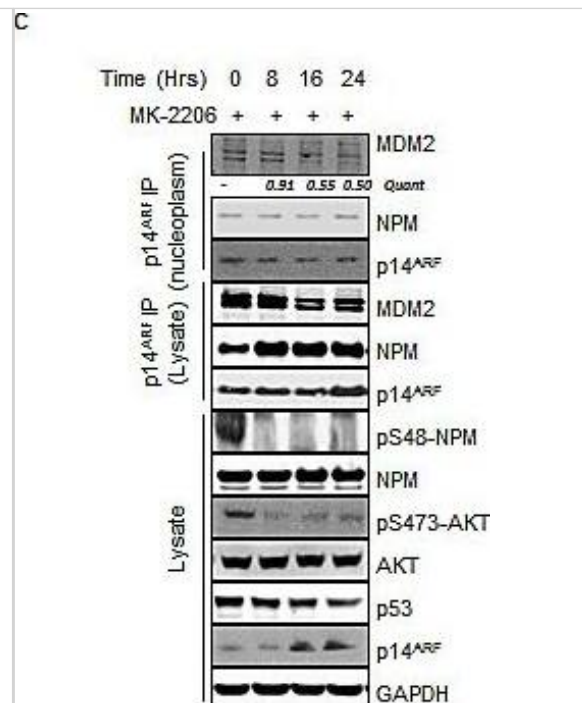
Immunocytochemistry/ Immunofluorescence: p14ARF/CDKN2A Antibody - BSA Free [NB200-111] - Inhibition of AKT modulates p53 stability in vivo & synergizes with ionizing radiation to inhibit tumor growth(A) PSN1 xenografts (PSN1 cells co-injected with LTC-14 stellate cells) established in the flank of athymic nude mice were treated with MK-2206 (60 mg/kg-320 mg/kg) as indicated or β -cyclo-dextrin (1.5 mg/ml) carrier. Xenograft tumors were lysed & lysates probed by western blot with the indicated antibodies. (B-D) Sections of PSN1 xenografts treated with three consecutive doses of MK-2206 (60 mg/kg). (B) Sections of PSN1 xenografts & in-vitro PSN1 cells fixed & stained with anti-NPM (red) & anti-p14ARF (green). (C) PSN1 xenografts treated with MK-2206 or carrier were stained with DAPI, anti-p53 (DO1) or p53mut (OP29 clone) (D) PSN1 xenografts treated with MK-2206 (60 mg/kg) or carrier were stained by immunohistochemical methods with anti-pS473-AKT, anti-phospho-S48-NPM (pS48-NPM) or p53. (E) PSN1 xenografts established in the flank of athymic nude mice were injected subcutaneously with two alternate day doses of MK-2206 (60 mg/kg) or carrier. Mice were subsequently treated with a single dose of IR (6 Gy) & tumor volumes measured regularly with callipers. Dash lines indicate tumor growth differential at 250 mm³. 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.



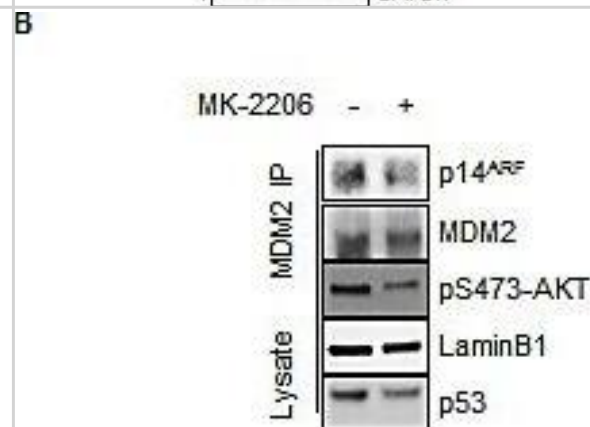
Western Blot: p14ARF/CDKN2A Antibody - BSA Free [NB200-111] - Inhibition of AKT decreases p53mut stability(A) T24 cells & PSN1 cells were treated with MK-2206 (5 μ M) or DMSO for 24 hrs. Cells were fixed & stained with DAPI & anti-mutant p53 (OP29 clone). (B) T24 cells were pre-treated with MK-2206 (5 μ M) or DMSO for 24 hrs before the addition of fresh media containing cyclohexamide (100 μ M) (CHX) in combination with MK-2206 (5 μ M) or DMSO for the times indicated. Nuclear extracts were prepared from treated cells & blotted with the indicated antibodies. Quantification is relative to initiation of CHX treatment for both conditions *, MK-2206 is 40% of DMSO control but taken as 1.0 for relative assessment. (C) H1299 cells were transfected with HA-tagged-ubiquitin & mutant p53 (R175H or R248W) as indicated. Transfected cells were treated with DMSO, MK-2206 (5 μ M) or Nutlin3A (5 μ M) for 16hrs as indicated. p53 immunoprecipitates & whole cell lysates were probed with the indicated antibodies. (D) T24 cells were transfected with non-targeting control or p14ARF siRNA & treated with DMSO, MK-2206 (5 μ M) or the NPM oligomerisation inhibitor NCS348884 (4 μ M) (Qi et al., 2008) as indicated. (E) T24 cells were transfected with non-targeting control, AKT1, or p14ARF siRNA. Cells were treated with NCS348884 (4 μ M), Nutlin3A (5 μ M) or DMSO as indicated. Whole cell lysates were probed with the indicated antibodies. (F) KPC mice derived KRASG12D p53 Floxed (p53Fl), KRASG12D p53R172H ARF+/+ & KRASG12D p53R172H ARF-/- pancreatic tumor cells were treated with MK-2206 (1 μ M), Nutlin3A (5 μ M) or DMSO as indicated. 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: p14ARF/CDKN2A Antibody - BSA Free [NB200-111] - 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. Immunopurification of NPM was done by pulling down with the Flag tag (middle panel) followed by elution of complexes by the Flag peptide & subsequent immunopurification 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.



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Publications

Kumar S, Song K, Wang J et al. Serum amyloid P secreted by bone marrow adipocytes drives skeletal amyloidosis. *Nature aging* 2025-07-29 [PMID: 40730660]

Ward C, Cauchy P, Garcia P et al. High WBP5 expression correlates with elevation of HOX genes levels and is associated with inferior survival in patients with acute myeloid leukaemia. *Scientific reports* 2020-11-24 [PMID: 32103106]

Xiong H, Yang Y, Yang K et al. Loss of the clock gene PER2 is associated with cancer development and altered expression of important tumor-related genes in oral cancer. *Int J Oncol* 2018-01-01 [PMID: 29115399] (Human)

Crawford Parks TE, Marcellus KA, Langill J et al. Novel Roles for Staufen1 in Embryonal and Alveolar Rhabdomyosarcoma via c-myc-dependent and -independent events. *Sci Rep.* 2017-02-17 [PMID: 28211476] (WB, Human)

Greiner T. Who chooses the leaders of UN organisations? *Lancet* 2010-03-27 [PMID: 20346814] (Human)

Magro PG, Russo AJ, Li WW et al. p14ARF expression increases dihydrofolate reductase degradation and paradoxically results in resistance to folate antagonists in cells with nonfunctional p53. *Cancer Res* 2004-06-15 [PMID: 15205349] (Human)

Hamilton Garth, Abraham Aswin G, Morton Jennifer et al. AKT regulates NPM dependent ARF localization and p53mut stability in tumors. *Oncotarget.* 2014-08-15 [PMID: 25071014] (ICC/IF)

Ghosh M, Ryan RO. Curcumin homing to the nucleolus: mechanism for initiation of an apoptotic program. *J Nutr. Biochem.* 2014-08-01 [PMID: 25172633] (WB, Human)

Williams RT, Barnhill LM, Kuo HH et al. Chimeras of p14ARF and p16: Functional Hybrids with the Ability to Arrest Growth. *PLoS ONE* 2014-02-07 [PMID: 24505435] (WB, Human)

Chen D, Shan J, Zhu WG, Qin J, Gu W et al. Transcription-independent ARF regulation in oncogenic stress-mediated p53 responses. *Nature* 2010-03-25 [PMID: 20208519] (IP, Human)

Chen D, Yoon JB, Gu W. Reactivating the ARF-p53 axis in AML cells by targeting ULF. *Cell Cycle* 2010-08-01 [PMID: 20699639] (IP, Human)

Muniz V, Barnes JM, Paliwal S et al. The ARF tumor suppressor inhibits tumor cell colonization independent of p53 in a novel mouse model of pancreatic ductal adenocarcinoma metastasis. *Mol Cancer Res* 9(7):867-77. 2011-07-01 [PMID: 21636682] (WB, Mouse)

More publications at <http://www.novusbio.com/NB200-111>

Procedures

Protocol specific for p14ARF Antibody (NB200-111)

Western Blot Procedure

- 1) Lyse HeLa or BT549 cells in Laemli buffer (2% SDS, 62.5 mM Tris pH 6.8, 10% glycerol, 5% 2-mercaptoethanol).
- 2) Boil the lysate for 5 minutes.
- 3) Load 25 ug of total cell extract, per lane, in a 15% SDS-PAGE minigel.
- 4) Transfer protein to PVDF membrane in 40 mM Tris base, 20 mM NaAcetate, 2 mM EDTA pH 7.4, 0.05% SDS, 20% methanol for 1 hour at 4 degrees C.
- 5) Rinse membrane in PBS-T and then block membrane in 5% nonfat dry milk/PBS-T (0.01M phosphate, 0.0027M KCl, 0.137M NaCl pH 7.4, 0.1% Tween-20) for 1 hour at room temperature or overnight at 4 degrees C.
- 6) Incubate membrane overnight at 4 degrees C with properly diluted NB200-111 (see data sheet) in PBS, 0.2% Tween-20, 5% nonfat dry milk.
- 7) Rinse the membrane 2X with 40 ml PBS-T. Wash membrane at room temperature, with 40 ml of PBS-T, 1X for 15 minutes and 2X for 5 minutes, each.
- 8) Incubate membrane with HRP conjugated anti-rabbit, diluted in PBS-T with 5% nonfat dry milk, for 1 hour at room temperature.
- 9) Wash membrane at room temperature, with 40 ml of PBS-T, 1X for 15 minutes and 4X for 5 minutes, each.
- 10) Develop with ECL reagents (Amersham) and autoradiography. Expose for 1+ minutes. For BT549 cells, 1 minute exposure is needed. For HeLa cells, additional exposure time may be required. In addition, a stronger ECL (Pierce) may be necessary.

NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.





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Limitations

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