Product Datasheet

PML Protein Antibody - BSA Free
NB100-59787

Unit Size: 0.1 mg

Store at 4C. Do not freeze.

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Updated 10/23/2024 v.20.1

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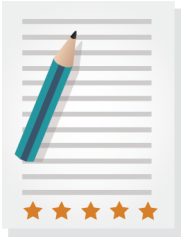


NB100-59787

PML Protein Antibody - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

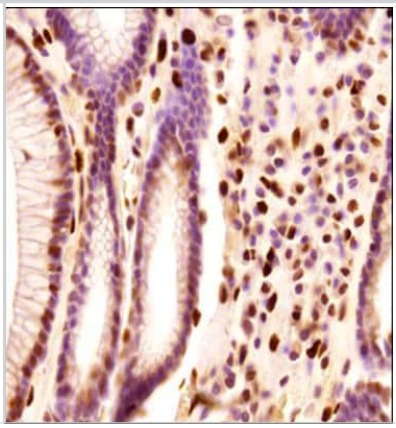
Host	Rabbit
Gene ID	5371
Gene Symbol	PML
Species	Human, Mouse
Reactivity Notes	Based on 100% sequence identity, this antibody is predicted to react with Orangutan, Gorilla, Chimpanzee, Primates, White-tufted-ear marmoset, Pygmy chimpanzee and Bornean orangutan. Mouse reactivity reported in scientific literature (PMID; 21343252)
Immunogen	Partial synthetic peptide made to an internal portion of human PML Protein (between amino acids 390-450) [P29590]

Product Application Details

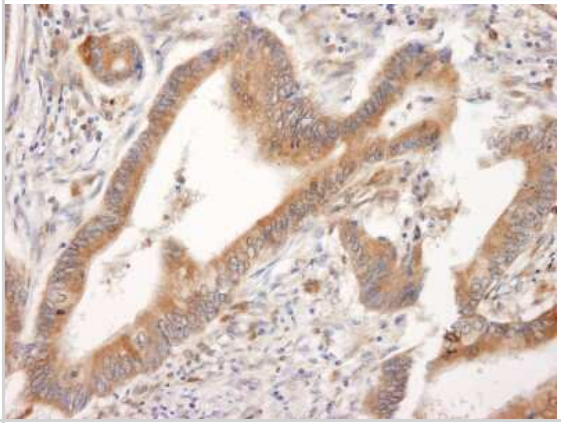
Applications	Western Blot, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Knockdown Validated
Recommended Dilutions	Western Blot 1:2000-1:10000, Flow Cytometry reported in scientific literature (PMID 28402725), Immunohistochemistry 1:200-1:1000, Immunocytochemistry/ Immunofluorescence 1:100-1:500, Immunoprecipitation 2-5 ug/mg lysate, Immunohistochemistry-Paraffin 1:200-1:1000, Immunoblotting reported in scientific literature (PMID 28239645), Proximity Ligation Assay 1:200-1:5000, Knockdown Validated reported in scientific literature (PMID 28402725)
Application Notes	Immunohistochemistry: Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.

Images

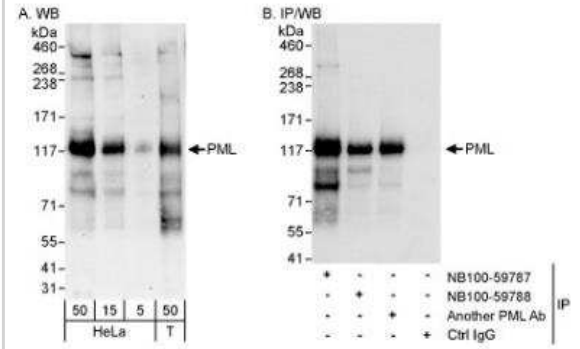
Immunohistochemistry-Paraffin: PML Protein Antibody [NB100-59787] - IHC analysis of a formalin fixed and paraffin embedded tissue section of human stomach using 1:50 dilution of PML antibody. The signal was developed using HRP-labeled secondary with DAB reagent followed by hematoxylin counterstaining. This antibody generated an expected strong nuclear staining in most of the cells in the glandular as well as the connective tissue. The cells showed some cytoplasmic signal also which was much weaker than the nuclear staining.



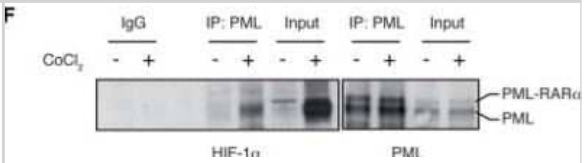
Immunohistochemistry: PML Protein Antibody [NB100-59787] - Sample: FFPE section of human prostate carcinoma. Antibody: Affinity purified rabbit anti- PML used at a dilution of 1:1,000 (0.2ug/ml). Detection: DAB



Western Blot: PML Protein Antibody [NB100-59787] - Detection of Human PML on HeLa whole cell lysate using NB100-59787. PML was also immunoprecipitated by rabbit anti-PML antibodies NB100-59788 and another CDC16/APC6 Ab.

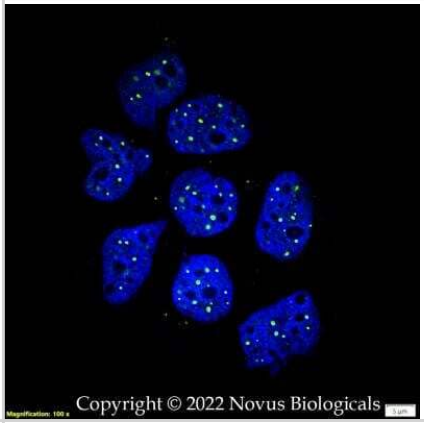


Western Blot: PML Protein Antibody [NB100-59787] - PML-RARa is a HIF-I+-transcriptional co-activator. HEK-293 cells transfected with stable mutants of HIF-1I+-or HIF-2I+-and increasing concentrations of PML-RARI+-. Image collected and cropped by Citeab from the following publication (HIF factors cooperate with PML-RAR +/- to promote acute promyelocytic leukemia progression and relapse. EMBO Mol Med (2014)) licensed under a CC-BY license.

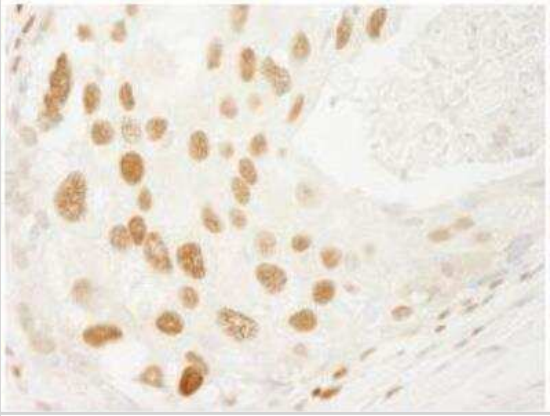


Immunocytochemistry/Immunofluorescence: PML Protein Antibody - BSA Free [NB100-59787] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with PML Protein Antibody (NB100-59787) at 2ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

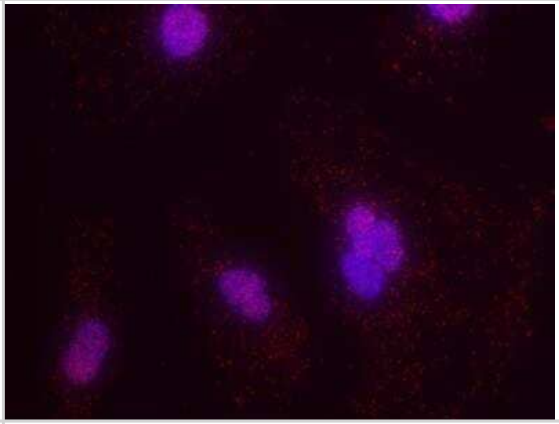
Immunocytochemistry/Immunofluorescence: PML Protein Antibody - BSA Free [NB100-59787] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with PML Protein Antibody (NB100-59787) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



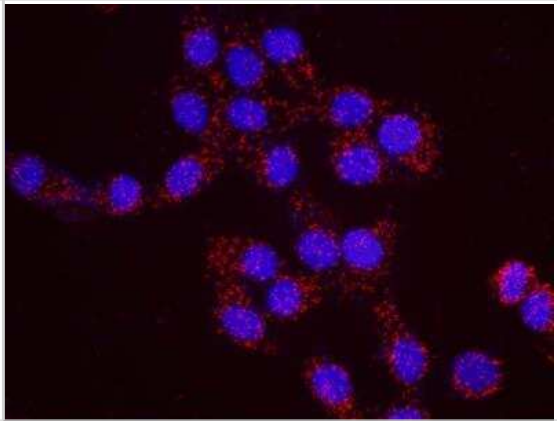
Immunohistochemistry-Paraffin: PML Protein Antibody [NB100-59787] - FFPE section of human breast carcinoma. Affinity purified rabbit anti-PML used at a dilution of 1:500



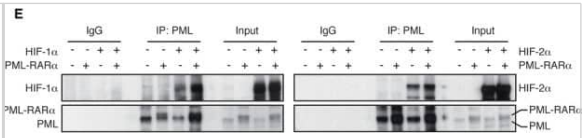
Proximity Ligation Assay: PML Protein Antibody [NB100-59787] - Secondary-conjugate Duolink II PLA in Hela cells. goat anti-human SMARCA4 (NBP2-22234) and rabbit anti-human PML (NB100-59787). Image merged from DAPI (2ms) and Texas Red (200ms) exposures, 40X magnification.



Proximity Ligation Assay: PML Protein Antibody [NB100-59787] - Secondary-conjugate Duolink II PLA in Hela cells. goat anti-human RFC4 (NB100-233) and rabbit anti-human PML (NB100-59787). Image merged from DAPI (2ms) and Texas Red (200ms) exposures, 40X magnification.



Western Blot: PML Protein Antibody - BSA Free [NB100-59787] - PML-RARa is a HIF-α transcriptional co-activator.A HIF-α transactivation assays (A–D) with HRE-luciferase construct. Results are presented as Luciferase/Renilla ratio (mean ± s.e.m. of experiments performed in triplicate).B HEK-293 cells transfected with stable mutants of HIF-1α or HIF-2α & increasing concentrations of PML-RARα.C HEK-293 cells transfected with a stable form of HIF-1α along with PML-RARα, PML or RARα.D Wild-type & Pml−/− MEFs transfected with HIF-1α & PML-RARα. Asterisks indicate fold change induction of HIF-1α-mediated transactivation upon PML-RARα expression.E HEK-293 cells transfected with the indicated fusion genes & treated with CoCl2.F Co-immunoprecipitation of exogenous stable forms of HIF-1α (left panel) & HIF-2α (right panel) & PML-RARα with a PML-directed antibody in HEK-293 cells. Of note, exogenously expressed PML-RARα migrates very closely to endogenous PML.Co-immunoprecipitation of endogenous HIF-1α & PML-RARα with a PML-directed antibody in NB4 cells treated with CoCl2.Data information: All experiments were repeated at least twice.Source data are available for this figure. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24711541), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

E Lång, A Po?e?, A Lång, M Valk, P Blicher, AD Rowe, KA Tønseth, CJ Jackson, TP Utheim, LMC Janssen, J Eriksson, SO Bøe Coordinated collective migration and asymmetric cell division in confluent human keratinocytes without wounding Nat Commun, 2018-09-10;9(1):3665. 2018-09-10 [PMID: 30202009]

Samuel Hofmann, Julius Luther, Verena Plank, Andreas Oswald, Julia Mai, Ilka Simons, Julija Miller, Valeria Falcone, Lea Hansen-Palmus, Hartmut Hengel, Michael Nassal, Ulrike Protzer, Sabrina Schreiner, Samuel K. Campos Arsenic trioxide impacts hepatitis B virus core nuclear localization and efficiently interferes with HBV infection Microbiology Spectrum 2024-05-01 [PMID: 38567974]

Fracassi C, Ugge' M, Abdelhalim M et al. PML modulates epigenetic composition of chromatin to regulate expression of pro-metastatic genes in triple-negative breast cancer Nucleic acids research 2023-10-12 [PMID: 37823593] (WB, Human)

Details:
1:1000 dilution

Hofmann S, Plank V, Groitl P et al. SUMO Modification of Hepatitis B Virus Core Mediates Nuclear Entry, Promyelocytic Leukemia Nuclear Body Association, and Efficient Formation of Covalently Closed Circular DNA Microbiology spectrum 2023-05-18 [PMID: 37199632]

Trier I, Black EM, Joo YK, Kabeche L ATR protects centromere identity by promoting DAXX association with PML nuclear bodies Cell reports 2023-05-30 [PMID: 37163376]

Göttig L, Weiß C, Stubbe M et al. Apobec3A Deamination Functions Are Involved in Antagonizing Efficient Human Adenovirus Replication and Gene Expression mBio 2023-05-08 [PMID: 37154747] (WB, Human)

Roos K, Berkholz J LDL Affects the Immunomodulatory Response of Endothelial Cells by Modulation of the Promyelocytic Leukemia Protein (PML) Expression via PKC International journal of molecular sciences 2023-04-15 [PMID: 37108469] (WB, Human)

Huang D, Zhao D, Li M et al. Crosstalk between PML and p53 in response to TGF-?1: A new mechanism of cardiac fibroblast activation International journal of biological sciences 2023-01-22 [PMID: 36778116] (PLA, Mouse)

Yan HY, Wang HQ, Zhong M et al. PML Suppresses Influenza Virus Replication by Promoting FBXW7 Expression Virologica Sinica 2021-05-27 [PMID: 34046815]

Lee J, Stone J, Desai P et al. Arsenicals, the Integrated Stress Response, and Epstein-Barr Virus Lytic Gene Expression Viruses 2021-04-30 [PMID: 33946406] (WB, Human)

Marks D, Heinen N, Bachmann L et al. Amyloid precursor protein elevates fusion of promyelocytic leukemia nuclear bodies in human hippocampal areas with high plaque load Acta neuropathologica communications 2021-04-13 [PMID: 33849647] (IF/IHC, Human)

Karle W, Becker S, Stenzel P et al. Promyelocytic leukemia protein (PML) promotes the phenotypic switch of smooth muscle cells in atherosclerotic plaques of human coronary arteries Clinical science (London, England : 1979) 2021-03-25 [PMID: 33764440]

More publications at http://www.novusbio.com/NB100-59787

Procedures

Western Blot Protocol for PML Protein Antibody (NB100-59787)
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 manufacturers instructions.

Immunocytochemistry/Immunofluorescence Protocol for PML Protein Antibody (NB100-59787)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for PML Protein Antibody (NB100-59787)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for PML Protein Antibody (NB100-59787)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NB100-59787

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB100-59787PEP	PML Protein Antibody Blocking Peptide

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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