abcam

Product datasheet

Anti-PML Protein antibody [EPR16792] ab179466

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-PML Protein antibody [EPR16792]
Description	Rabbit monoclonal [EPR16792] to PML Protein
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 293T, K562, HeLa and A549 whole cell lysates; Human fetal brain, fetal heart and fetal kidney lysates; HAP1. IHC-P: Human mammary gland and breast carcinoma tissues. ICC/IF: K562 cells. ICC/IF KO: Hap1 cells (Hap1-PML KO used as a negative cell line). IP: K562 whole cell extract.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16792

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab179466 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
IP		1/70.
ICC/IF	★★★★★ (3)	1/500.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★☆ (2)	1/1000 - 1/2000. Detects a band of approximately 50-110 kDa (predicted molecular weight: 98 kDa).

Target

Function Key component of PML nuclear bodies that regulate a large number of cellular processes by facilitating post-translational modification of target proteins, promoting protein-protein contacts, or by sequestering proteins. Functions as tumor suppressor. Required for normal, caspase-dependent apoptosis in response to DNA damage, FAS, TNF, or interferons. Plays a role in transcription regulation, DNA damage response, DNA repair and chromatin organization. Plays a role in processes regulated by retinoic acid, regulation of cell division, terminal differentiation of myeloid precursor cells and differentiation of neural progenitor cells. Required for normal immunity to microbial infections. Plays a role in antiviral response. In the cytoplasm, plays a role in TGFB1-dependent processes. Regulates p53/TP53 levels by inhibiting its ubiquitination and proteasomal degradation. Regulates activation of p53/TP53 via phosphorylation at 'Ser-20'. Sequesters MDM2 in the nucleolus after DNA damage, and thereby inhibits ubiquitination and degradation of p53/TP53. Regulates translation of HIF1A by sequestering MTOR, and thereby plays a role in neoangiogenesis and tumor vascularization. Regulates RB1 phosphorylation and activity. Required for normal development of the brain cortex during embryogenesis. Can sequester herpes virus and varicella virus proteins inside PML bodies, and thereby inhibit the formation of infectious viral particles. Regulates phosphorylation of ITPR3 and plays a role in the regulation of calcium homeostasis at the endoplasmic reticulum (By similarity). Regulates transcription activity of ELF4. Inhibits specifically the activity of the tetrameric form of PKM2. Together with SATB1, involved in local chromatin-loop remodeling and gene expression regulation at the MHC-I locus. Regulates PTEN compartmentalization through the inhibition of USP7-mediated deubiquitinylation.

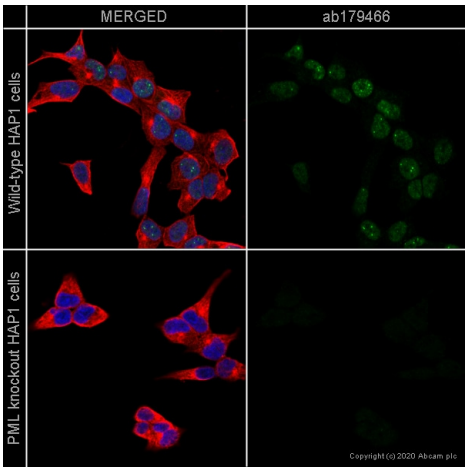
Involvement in disease Note=A chromosomal aberration involving PML may be a cause of acute promyelocytic leukemia (APL). Translocation t(15;17)(q21;q21) with RARA. The PML breakpoints (type A and type B) lie on either side of an alternatively spliced exon.

Sequence similarities Contains 2 B box-type zinc fingers.
Contains 1 RING-type zinc finger.

Domain Interacts with PKM2 via its coiled-coil domain.
Binds arsenic via the RING-type zinc finger.

Post-translational modifications	Ubiquitinated; mediated by RNF4, SIAH1 or SIAH2 and leading to subsequent proteasomal degradation. 'Lys-6'-, 'Lys-11'-, 'Lys-48'- and 'Lys-63'-linked polyubiquitination by RNF4 is polysumoylation-dependent. Undergoes 'Lys-11'-linked sumoylation. Sumoylation on all three sites is required for nuclear body formation. Sumoylation on Lys-160 is a prerequisite for sumoylation on Lys-65. The PML-RARA fusion protein requires the coiled-coil domain for sumoylation. Desumoylated by SENP2 and SENP6. Arsenic induces PML and PML-RARA oncogenic fusion proteins polysumoylation and their subsequent RNF4-dependent ubiquitination and proteasomal degradation, and is used as treatment in acute promyelocytic leukemia (APL). Phosphorylated in response to DNA damage, probably by ATR. Acetylation may promote sumoylation and enhance induction of apoptosis.
Cellular localization	Nucleus > nucleoplasm. Cytoplasm. Nucleus > PML body. Nucleus > nucleolus. Endoplasmic reticulum membrane. Early endosome membrane. Sumoylated forms localize to the PML nuclear bodies. The B1 box and the RING finger are also required for this nuclear localization. Isoforms lacking a nuclear localization signal are cytoplasmic. Detected in the nucleolus after DNA damage. Sequestered in the cytoplasm by interaction with rabies virus phosphoprotein.

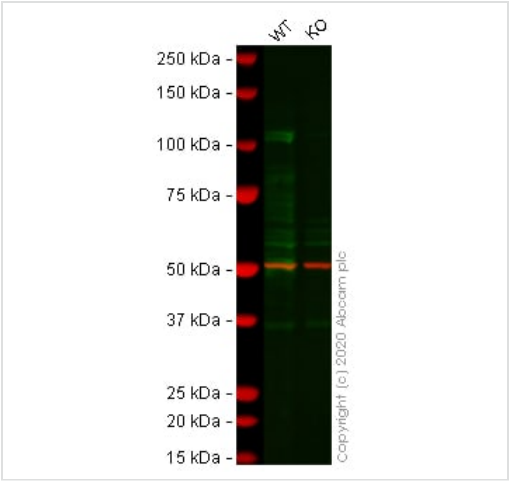
Images



Immunocytochemistry/ Immunofluorescence - Anti-PML Protein antibody [EPR16792] (ab179466)

ab179466 staining PML in wild-type Hap1 cells (top panel) and PML knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), 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 with ab179466 at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-PML Protein antibody [EPR16792] (ab179466)

All lanes : Anti-PML Protein antibody [EPR16792] (ab179466) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : PML knockout A549 cell lysate

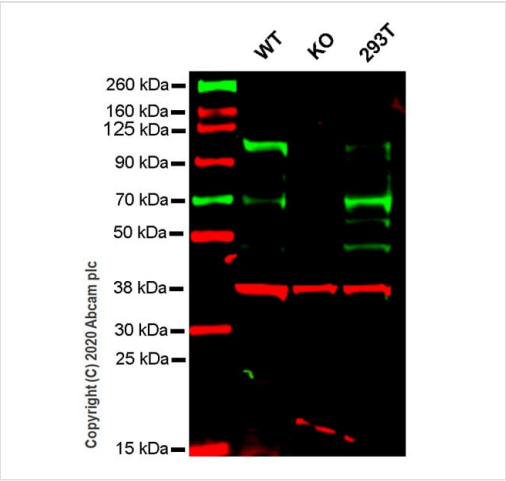
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 98 kDa
Observed band size: 110 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab179466 observed at 110 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab179466 was shown to react with PML in A549 wild-type cells in western blot with loss of signal observed in PML knockout cell line **ab266980** (PML knockout cell lysate **ab257082**). Wild-type and PML knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk before incubation with ab179466 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-PML Protein antibody [EPR16792] (ab179466)

All lanes : Anti-PML Protein antibody [EPR16792] (ab179466) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PML knockout HeLa cell lysate
Lane 3 : 293T cell lysate

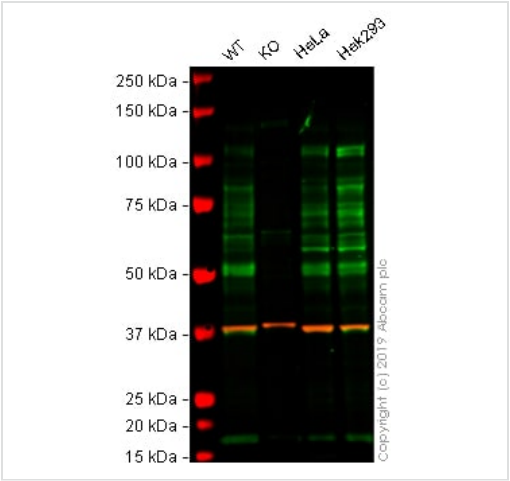
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 98 kDa
Observed band size: 110 kDa

Lanes 1-3: Merged signal (red and green). Green - ab179466 observed at 50-110 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab179466 Anti-PML Protein antibody [EPR16792] was shown to specifically react with PML Protein in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261811** (knockout cell lysate **ab257081**) was used. Wild-type and PML Protein knockout samples were subjected to SDS-PAGE. ab179466 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PML Protein antibody [EPR16792] (ab179466)

All lanes : Anti-PML Protein antibody [EPR16792] (ab179466) at 1/1000 dilution

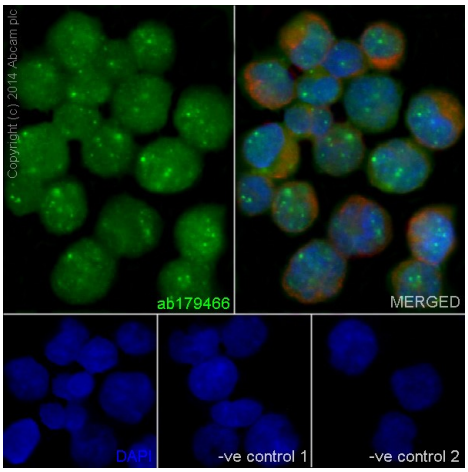
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : PML knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 98 kDa
Observed band size: 50-110 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab179466 observed at 50-110 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab179466 was shown to recognize in wild-type HAP1 cells as signal was lost at the expected MW in PML knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and PML knockout samples were subjected to SDS-PAGE. Ab179466 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

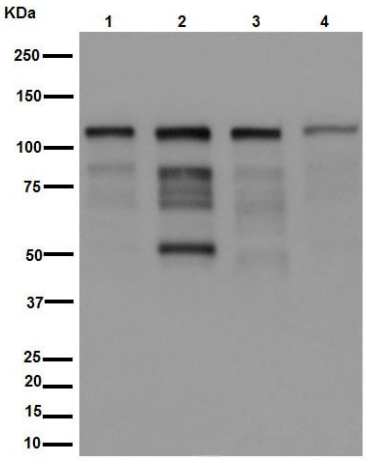


Immunocytochemistry/ Immunofluorescence - Anti-PML Protein antibody [EPR16792] (ab179466)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling PML Protein with ab179466 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Cytoplasm and nuclear staining on K562 cell line is observed. The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-
-ve control 1 - ab179466 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2. - **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution

followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



Western blot - Anti-PML Protein antibody [EPR16792] (ab179466)

All lanes : Anti-PML Protein antibody [EPR16792] (ab179466) at 1/20000 dilution

Lane 1 : 293T (Human epithelial cells from embryonic kidney) whole cell lysate
Lane 2 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate
Lane 3 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 4 : A549 (Human lung carcinoma) whole cell lysate

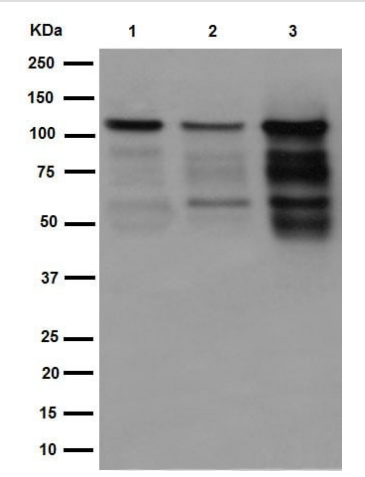
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 98 kDa
Observed band size: 50-110 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

ab179466 recognizes 12 isoforms shown as multiple bands ranging from 50 kDa to 110 kDa.



Western blot - Anti-PML Protein antibody [EPR16792] (ab179466)

All lanes : Anti-PML Protein antibody [EPR16792] (ab179466) at 1/2000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate

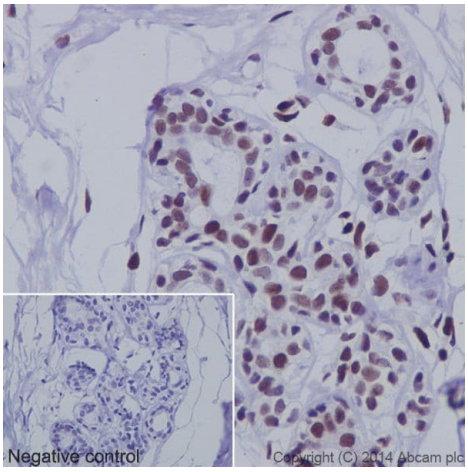
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 98 kDa
Observed band size: 50-110 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

ab179466 recognizes 12 isoforms shown as multiple bands ranging from 50 kDa to 110 kDa.

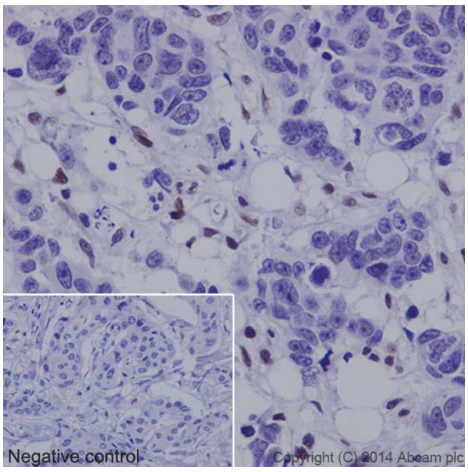


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PML Protein antibody [EPR16792] (ab179466)

Immunohistochemical analysis of paraffin-embedded Human mammary gland tissue labeling PML Protein with ab179466 at 1/2000 dilution followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus staining on epithelial cells of Human mammary gland tissue is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



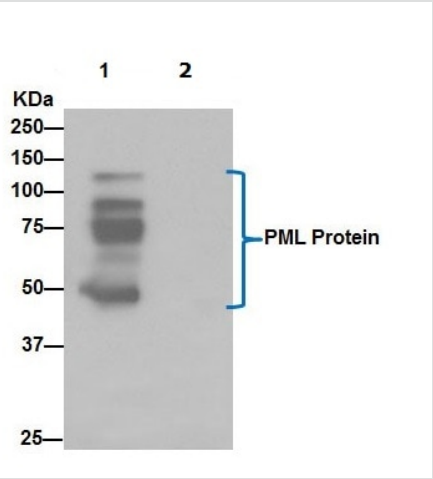
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PML Protein antibody [EPR16792] (ab179466)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling PML Protein with ab179466 at 1/2000 dilution followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Counter stained with Hematoxylin. The breast cancer cells lost expression.

Reference: Gurrieri C et al. Loss of the Tumor Suppressor PML in Human Cancers of Multiple Histologic Origins. J Natl Cancer Inst 96:269 –279 (2004).

Negative control: Used PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

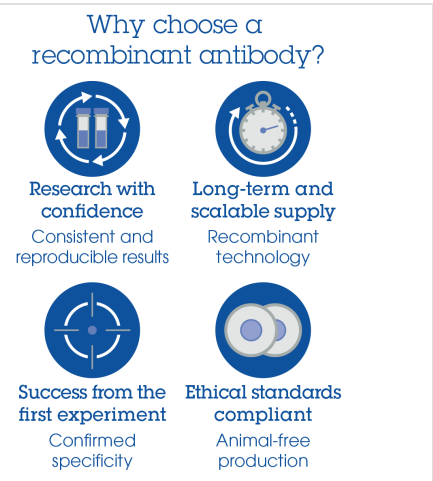


Immunoprecipitation - Anti-PML Protein antibody [EPR16792] (ab179466)

PML Protein was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell extract with ab179466 at 1/70 diltuion. Western blot was performed from the immunoprecipitate using ab179466 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. Lane 1: K562 whole cell extract. Lane 2: PBS instead of K562 whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

ab179466 recognizes 12 isoforms shown as multiple bands ranging from 50 kDa to 110 kDa.



Anti-PML Protein antibody [EPR16792] (ab179466)

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