

Product datasheet

Human PML (PML Protein) knockout HeLa cell lysate ab257081

2 Images

Overview

Product name	Human PML (PML Protein) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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Tested applications

Suitable for: WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260904 - Human PML knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 WWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

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 deubiquitylation.

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 Ubiquitinated; mediated by RNF4, SIAH1 or SIAH2 and leading to subsequent proteasomal

modifications

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.

Applications

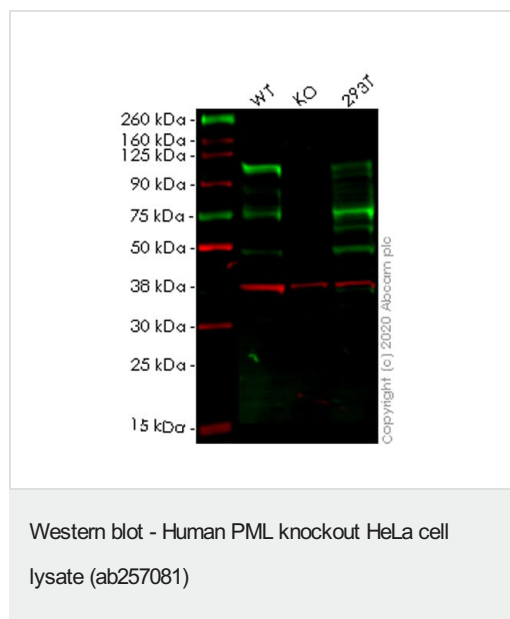
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab257081 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 98 kDa.

Images



Lane 1: Wild-type HeLa cell lysate (20 µg)

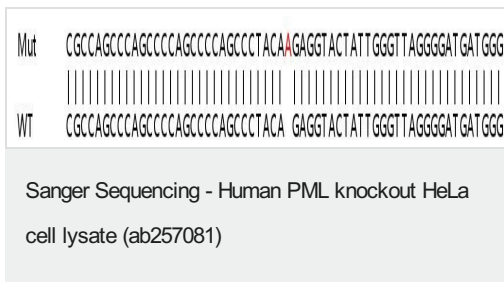
Lane 2: PML knockout HeLa cell lysate (20 µg)

Lane 3: 293T cell lysate (20 µg)

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.



Homozygous: 1 bp insertion in exon 1

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