# abcam

### **Product datasheet**

## Human PML (PML Protein) knockout HeLa cell line ab261811

画像数 2

製品の概要

| 製品名                  | Human PML (PML Protein) knockout HeLa cell line  |  |  |
|----------------------|--|--|--|
| 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)   |  |  |
| アプリケーション             | <b>適用あり:</b> WB  |  |  |
| Biosafety level      | 2  |  |  |
| 特記事項                 | <b>Recommended control:</b> Human wild-type HeLa cell line ( <u>ab255928</u> ). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.   |  |  |
|                      | <b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.   |  |  |
|                      | Culture medium: DMEM (High Glucose) + 10% FBS  |  |  |
|                      | <b>Initial handling guidelines:</b> Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.   |  |  |
|                      | <ol> <li>Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.</li> <li>Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10<sup>4</sup> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> </ol> |  |  |
|                      | 4. Incubate the culture at 37°C incubator with 5% CO <sub>2</sub> . Cultures should be monitored daily.  |  |  |
|                      | <b>Subculture guidelines:</b><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of 2x10 <sup>4</sup> cells/cm <sup>2</sup> is recommended.<br>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if<br>required.  |  |  |

Cells should be passaged when they have achieved 80-90% confluence. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

#### 製品の特性

| Number of cells       | 1 x 10 <sup>6</sup> cells/vial, 1 mL  |  |
|-----------------------|---|--|
| Adherent /Suspension  | Adherent  |  |
| Tissue                | Cervix  |  |
| Cell type             | epithelial  |  |
| Disease               | Adenocarcinoma  |  |
| Gender                | Female  |  |
| STR Analysis          | Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18<br>TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10 |  |
| Antibiotic resistance | Puromycin 1.00µg/ml   |  |
| Mycoplasma free       | Yes   |  |
| 保存方法                  | Shipped on Dry Ice. Store in liquid nitrogen.   |  |
| バッファー                 | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether  |  |

#### ターゲット情報

#### 機能

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, caspasedependent 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 TGFB1dependent 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 deubiguitinylation.

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.  |  |
|-------|---|--|
| 配列類似性 | Contains 2 B box-type zinc fingers.<br>Contains 1 RING-type zinc finger.  |  |
| ドメイン  | Interacts with PKM2 via its coiled-coil domain.<br>Binds arsenic via the RING-type zinc finger.   |  |
| 翻訳後修飾 | 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.<br>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).<br>Phosphorylated in response to DNA damage, probably by ATR.<br>Acetylation may promote sumoylation and enhance induction of apoptosis. |  |
| 細胞内局在 | Nucleus > nucleoplasm. Cytoplasm. Nucleus > PML body. Nucleus > nucleolus. Endoplasmic<br>reticulum membrane. Early endosome membrane. Sumoylated forms localize to the PML nuclear<br>bodies. The B1 box and the RING finger are also required for this nuclear localization. Isoforms<br>lacking a nuclear localization signal are cytoplasmic. Detected in the nucleolus after DNA<br>damage. Sequestered in the cytoplasm by interaction with rabies virus phosphoprotein.  |  |

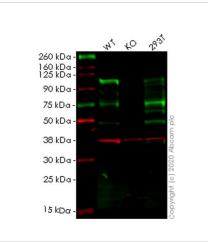
アプリケーション

 The Abpromise guarantee
 Abpromise保証は、次のテスト済みアプリケーションにおけるab261811の使用に適用されます

 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項   |
|----------|-----------|--|
| WB       |           | Use at an assay dependent concentration. Predicted molecular weight: 98 kDa. |

画像



Western blot - Human PML knockout HeLa cell line (ab261811)

All lanes : Anti-PML Protein antibody [EPR16792] (<u>ab179466</u>) 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.

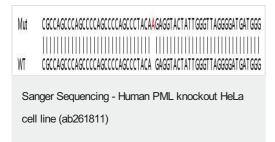
Performed under reducing conditions.

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

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

<u>ab179466</u> 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 <u>ab257081</u>) was used. Wild-type and PML Protein knockout samples were subjected to SDS-PAGE. <u>ab179466</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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<sup>®</sup> 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) 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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