abcam

Product datasheet

Human PTEN knockout HeLa cell line ab255419

画像数 6

製品の概要

製品名	Human PTEN knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 11 bp deletion in exon 5 and 5 bp insertion in exon 5 and Insertion of the selection cassette in exon 5
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** 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. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. 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. 3. 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 $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

required.
Cells should be passaged when they have achieved 80-90% confluence.
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We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 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 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
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	Tumor suppressor. Acts as a dual-specificity protein phosphatase, dephosphorylating tyrosine-, serine- and threonine-phosphorylated proteins. Also acts as a lipid phosphatase, removing the phosphate in the D3 position of the inositol ring from phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-diphosphate, phosphatidylinositol 3-phosphate and inositol 1,3,4,5-tetrakisphosphate with order of substrate preference in vitro PtdIns(3,4,5)P3 > PtdIns(3,4)P2 > PtdIns3P > Ins(1,3,4,5)P4. The lipid phosphatase activity is critical for its tumor suppressor function. Antagonizes the PI3K-AKT/PKB signaling pathway by dephosphorylating phosphoinositides and thereby modulating cell cycle progression and cell survival. The unphosphorylated form cooperates with AIP1 to suppress AKT1 activation. Dephosphorylates tyrosine-phosphorylated focal adhesion kinase and inhibits cell migration and integrin-mediated cell spreading and focal adhesion formation. Plays a role as a key modulator of the AKT-mTOR signaling pathway controlling the tempo of the process of newborn neurons integration during adult neurogenesis, including correct neuron positioning, dendritic development and synapse formation. May be a negative regulator of insulin signaling and glucose metabolism in adipose tissue. The nuclear monoubiquitinated form possesses greater apoptotic potential, whereas the cytoplasmic nonubiquitinated form induces less tumor suppressive ability. In motile cells, suppresses the formation of lateral pseudopods and thereby promotes cell polarization and directed movement. Isoform alpha: Functional kinase, like isoform 1 it antagonizes the PI3K-AKT/PKB signaling pathway. Plays a role in mitochondrial energetic metabolism by promoting COX activity and ATP production, via collaboration with isoform 1 in increasing protein levels of PINK1.
組織特異性	Expressed at a relatively high level in all adult tissues, including heart, brain, placenta, lung, liver,

	muscle, kidney and pancreas.
関連疾患	Cowden syndrome 1 Lhermitte-Duclos disease Bannayan-Riley-Ruvalcaba syndrome Squamous cell carcinoma of the head and neck Endometrial cancer PTEN mutations are found in a subset of patients with Proteus syndrome, a genetically heterogeneous condition. The molecular diagnosis of PTEN mutation positive cases classifies Proteus syndrome patients as part of the PTEN hamartoma syndrome spectrum. As such, patients surviving the early years of Proteus syndrome are likely at a greater risk of developing malignancies. Glioma 2 VACTERL association with hydrocephalus Prostate cancer Macrocephaly/autism syndrome A microdeletion of chromosome 10q23 involving BMPR1A and PTEN is a cause of chromosome 10q23 deletion syndrome, which shows overlapping features of the following three disorders: Bannayan-Zonana syndrome, Cowden disease and juvenile polyposis syndrome.
配列類似性	Contains 1 C2 tensin-type domain. Contains 1 phosphatase tensin-type domain.
ドメイン	The C2 domain binds phospholipid membranes in vitro in a Ca(2+)-independent manner; this binding is important for its tumor suppressor function.
翻訳後修飾	Constitutively phosphorylated by CK2 under normal conditions. Phosphorylated in vitro by MAST1, MAST2, MAST3 and STK11. Phosphorylation results in an inhibited activity towards PIP3. Phosphorylation can both inhibit or promote PDZ-binding. Phosphorylation at Tyr-336 by FRK/PTK5 protects this protein from ubiquitin-mediated degradation probably by inhibiting its binding to NEDD4. Phosphorylation by ROCK1 is essential for its stability and activity. Phosphorylation by PLK3 promotes its stability and prevents its degradation by the proteasome. Monoubiquitinated; monoubiquitination is increased in presence of retinoic acid. Deubiquitinated by USP7; leading to its nuclear exclusion. Monoubiquitination of one of either Lys-13 and Lys-289 amino acid is sufficient to modulate PTEN compartmentalization. Ubiquitinated by XIAP/BIRC4.
細胞内局在	Secreted. May be secreted via a classical signal peptide and reenter into cells with the help of a poly-Arg motif and Cytoplasm. Nucleus. Nucleus, PML body. Monoubiquitinated form is nuclear. Nonubiquitinated form is cytoplasmic. Colocalized with PML and USP7 in PML nuclear bodies. XIAP/BIRC4 promotes its nuclear localization.

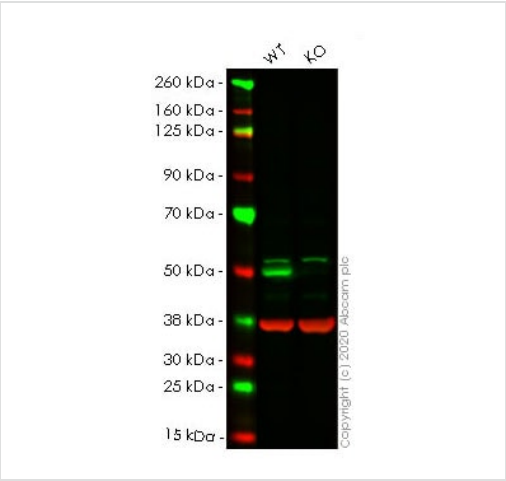
アプリケーション

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ab255419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: 47 kDa.

画像



Western blot - Human PTEN knockout HeLa cell line (ab255419)

All lanes : Anti-PTEN antibody [EPR9941] (**ab154812**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PTEN knockout HeLa cell lysate

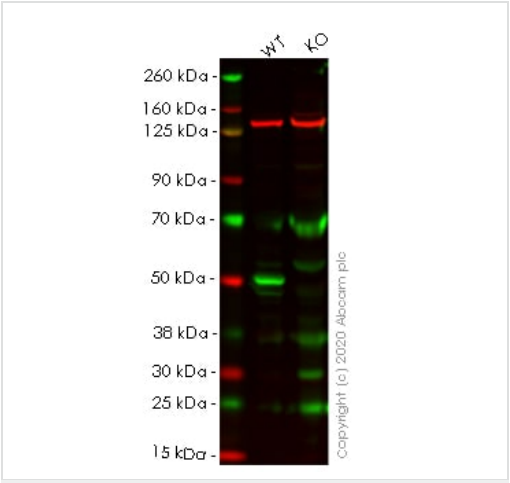
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab154812** observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab154812 was shown to react with PTEN in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255419 (knockout cell lysate **ab263829**) was used. Wild-type HeLa and PTEN knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab154812** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human PTEN knockout HeLa cell line (ab255419)

All lanes : Anti-PTEN antibody [EPR4408-76] (**ab133532**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PTEN knockout HeLa cell lysate

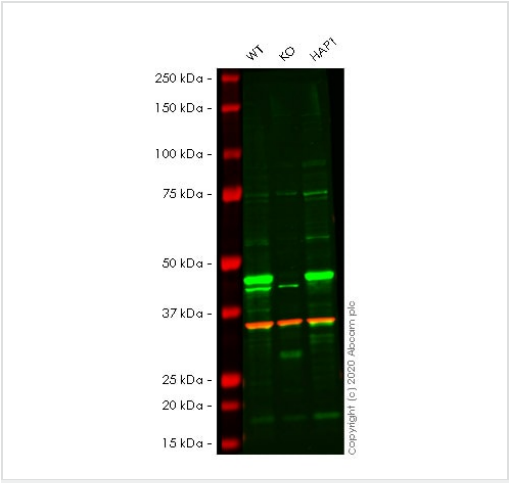
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab133532** observed at 47 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab133532 was shown to react with PTEN in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255419 (knockout cell lysate **ab263829**) was used. Wild-type HeLa and PTEN knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab133532** and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 10000 dilution and a 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human PTEN knockout HeLa cell line (ab255419)

All lanes : Anti-PTEN antibody [EPR4408-76] (**ab133532**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PTEN knockout HeLa cell lysate
Lane 3 : HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab133532** observed at 47 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

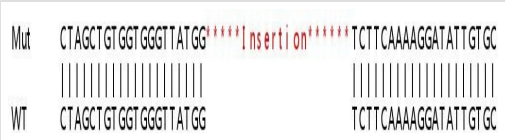
ab133532 was shown to react with PTEN in wild-type HeLa cells in western blot. The bands observed in PTEN knockout cell line ab255419 (PTEN knockout cell lysate **ab263829**) below 47 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. HeLa wild-type and PTEN knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab133532** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 10000 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.

Sanger Sequencing - Human PTEN knockout HeLa cell line (ab255419)

Allele-1: 11 bp deletion in exon 5.

Sanger Sequencing - Human PTEN knockout HeLa cell line (ab255419)

Allele-2: 5 bp insertion in exon 5.



Sanger Sequencing - Human PTEN knockout HeLa cell line (ab255419)

Allele-3: Insertion of the selection cassette in exon 5.

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