abcam

Product datasheet

Human AXL knockout HeLa cell line ab261810

4 Images

Overview

Product name	Human AXL knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 4 and 4 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	May function as a signal transducer between specific cell types of mesodermal origin. In case of filovirus infection, seems to function as a cell entry factor.
Tissue specificity	Highly expressed in metastatic colon tumors. Expressed in primary colon tumors. Weakly expressed in normal colon tissue.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. AXL/UFO subfamily. Contains 2 fibronectin type-III domains. Contains 2 Ig-like C2-type (immunoglobulin-like) domains. Contains 1 protein kinase domain.
Cellular localization	Membrane.

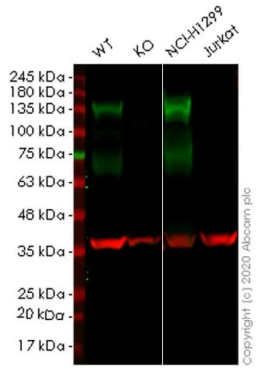
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261810 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



Western blot - Human AXL knockout HeLa cell line (ab261810)

All lanes : Anti-Axl antibody [EPR23892-15] (**ab259831**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : Axl knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : NCI-H1299 (human lung carcinoma epithelial cell) whole cell lysate
Lane 4 : Jurkat (human T cell leukemia T lymphocyte), whole cell lysate

Lysates/proteins at 40 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

Predicted band size: 98 kDa
Observed band size: 140,80 kDa

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

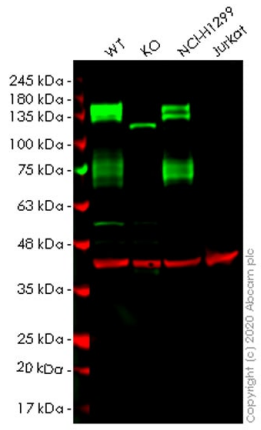
Lanes 1-4: Merged signal (red and green). Green - **ab259831** observed at 140, 80 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab259831 Anti-Axl antibody [EPR23892-15] was shown to react with Axl in Hela cells in Western blot. Loss of signal was observed when knockout cell line ab261810 (knockout cell lysate **ab257151**) was used. Wild-type and Axl knockout samples were subjected to SDS-PAGE.

ab259831 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight 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.

Negative control: Jurkat (PMID: 28423548).



Western blot - Human AXL knockout HeLa cell line (ab261810)

All lanes : Anti-Axl antibody [EPR21107] (**ab215205**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : AXL knockout HeLa cell lysate
Lane 3 : NCI-H1299 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 98 kDa
Observed band size: 80,140 kDa

Western blot: Anti-Axl antibody [EPR21107] (**ab215205**) staining at 1:1000 dilution, shown in green; Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) shown in red.

ab215205 was shown to bind specifically to Axl. A band was observed at 140 kDa in wild-type HeLa cell lysates with no signal observed at this size in Axl CRISPR-Cas9 edited cell line ab261810 (CRISPR-Cas9 edited cell lysate **ab257151**). The band observed in the CRISPR-Cas9 edited lysate lane below 90 kDa is likely to represent a truncated form of Axl. This has not been investigated further and the functional properties of the gene product have not been determined.

Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/10000 dilution.

Sanger Sequencing - Human AXL knockout HeLa cell line (ab261810)

Allele-1: 1 bp deletion in exon 4.

Sanger Sequencing - Human AXL knockout HeLa cell line (ab261810)

Allele-2: 4 bp deletion in exon 4.

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