abcam

Product datasheet

Human AXL knockout HeLa cell lysate ab257151

4 Images

Overview

Product name	Human AXL 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, 1 bp deletion in exon4 and 4 bp deletion in exon4.
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. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
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.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab260903 - Human AXL 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 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

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.

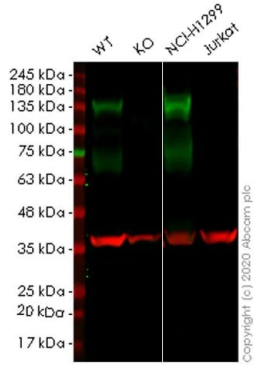
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257151 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 lysate (ab257151)

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

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

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

Lane 3: NCI-H1299 cell lysate (20 µg)

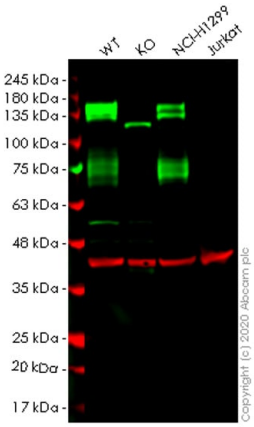
Lane 4: Jurkat cell lysate (20 µg)

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 lysate (ab257151)

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

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Lane 3: NCI-H1299 cell lysate (20 µg)

Lane 4: Jurkat cell lysate (20 µg)

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 lysate (ab257151)

Allele-1: 1 bp deletion in exon4

Sanger Sequencing - Human AXL knockout HeLa cell lysate (ab257151)

Allele-2: 4 bp deletion in exon4

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