

Product datasheet

Human PODXL knockout HCT116 cell lysate ab257211

2 Images

Overview

Product name	Human PODXL knockout HCT116 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon2.
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
ab263498 - Human PODXL knockout HCT116 cell lysate	1 x 100µg
ab255555 - Human wild-type HCT116 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

STR Analysis Amelogenin X D5S818: 10, 11 D13S317: 10, 12 D7S820: 11, 12 D16S539: 11, 13 vWA: 17, 22 TH01: 8,9 TPOX: 8, 9 CSF1PO: 7, 10

Target

Function Involved in the regulation of both adhesion and cell morphology and cancer progression. Function as an anti-adhesive molecule that maintains an open filtration pathway between neighboring foot processes in the podocyte by charge repulsion. Acts as a pro-adhesive molecule, enhancing the adherence of cells to immobilized ligands, increasing the rate of migration and cell-cell contacts in an integrin-dependent manner. Induces the formation of apical actin-dependent microvilli. Involved in the formation of a preapical plasma membrane subdomain to set up initial epithelial polarization and the apical lumen formation during renal tubulogenesis. Plays a role in cancer development and aggressiveness by inducing cell migration and invasion through its interaction with the actin-binding protein EZR. Affects EZR-dependent signaling events, leading to increased activities of the MAPK and PI3K pathways in cancer cells.

Tissue specificity Glomerular epithelium cell (podocyte).

Sequence similarities Belongs to the podocalyxin family.

Domain Both the O-glycan-rich domain of the extracellular domain and the C-terminus PDZ-binding motif (DTHL) in the cytoplasmic tail harbor an apical sorting signal. The cytoplasmic domain is necessary for the apical membrane targeting and renal tubulogenesis. The cytoplasmic C-terminus PDZ-binding motif (DTHL) is essential for interaction with SLC9A3R1 and for targeting SLC9A3R1 to the apical cell membrane. The extracellular domain is necessary for microvillus formation (By similarity). The large highly anionic extracellular domain allows to maintain open filtration pathways between neighboring podocyte foot processes.

Post-translational modifications N- and O-linked glycosylated. Sialoglycoprotein.

Cellular localization Apical cell membrane. Cell projection, lamellipodium. Cell projection, filopodium. Cell projection, ruffle. Cell projection, microvillus. Membrane raft. Membrane. In single attached epithelial cells is restricted to a preapical pole on the free plasma membrane whereas other apical and basolateral proteins are not yet polarized. Colocalizes with SLC9A3R2 at the apical plasma membrane during epithelial polarization. Colocalizes with SLC9A3R1 at the trans-Golgi network (transiently) and at the apical plasma membrane. Its association with the membrane raft is transient. Colocalizes with actin filaments, EZR and SLC9A3R1 in a punctate pattern at the apical cell surface where microvilli form. Colocalizes with EZR and SLC9A3R2 at the apical cell membrane of glomerular epithelium cells (By similarity). Forms granular, punctuated pattern, forming patches, preferentially adopting a polar distribution, located on the migrating poles of the cell or forming clusters along the terminal ends of filipodia establishing contact with the endothelial cells.

Colocalizes with the submembrane actin of lamellipodia, particularly associated with ruffles. Colocalizes with vinculin at protrusions of cells. Colocalizes with ITGB1. Colocalizes with PARD3, PRKCI, EXOC5, OCLN, RAB11A and RAB8A in apical membrane initiation sites (AMIS) during the generation of apical surface and lumenogenesis (By similarity).

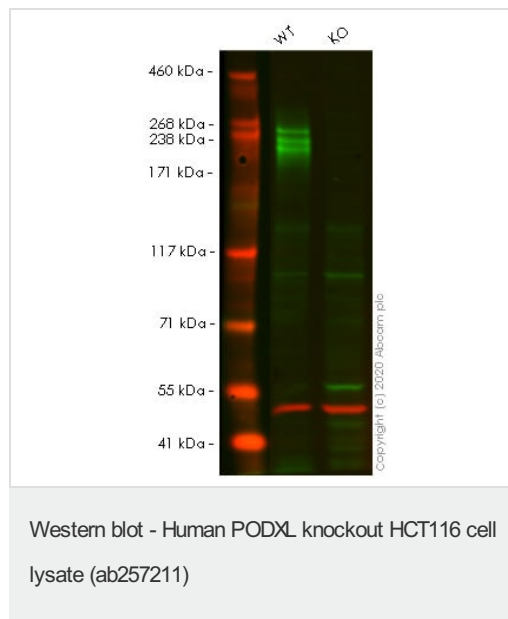
Form There are 2 isoforms produced by alternative splicing.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab257211 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: 58 kDa.

Images



Lane 1: Wild-type HCT116 cell lysate (20µg)

Lane 2: PODXL knockout HCT116 cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - [ab150358](#) observed at 200 kDa. Red - loading control [ab7291](#) observed at 50 kDa.

[ab150358](#) Anti-PODXL antibody [EPR9518] was shown to specifically react with PODXL in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line [ab266887](#) (knockout cell lysate ab257211) was used. Wild-type and PODXL knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. [ab150358](#) and Anti-alpha Tubulin antibody [DM1A] - Loading Control ([ab7291](#)) 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 20000 dilution for 1 hour at room temperature before imaging.

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Mut  ACTTGCTGAGCCAGGGTTGTAGTCCCCGGT GAGTCACTGGATACACCAAGGGTGGTCGC
      |||
WT   ACTTGCTGAGCCAGGGTTGTAGTCCCCGGT GAGTCACTGGATACACCAAGGGTGGTCGC
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Sanger Sequencing - Human PODXL knockout
HCT116 cell lysate (ab257211)

Homozygous: 1 bp insertion in exon2

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