

Human PODXL knockout HCT116 cell line ab266887

画像数 3

製品の概要

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|-----------------------------|---|
| 製品名 | Human PODXL knockout HCT116 cell line |
| Parental Cell Line | HCT116 |
| Organism | Human |
| Mutation description | Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 2 |
| 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 | 1 |
| 特記事項 | <p>Recommended control: Human wild-type HCT116 cell line (ab255451). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: McCoY5a + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p> |

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 | Colon |
| Cell type | epithelial |
| Disease | Carcinoma |
| Gender | Male |
| 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 |
| 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

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|--------------|---|
| 機能 | 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. |
| 組織特異性 | Glomerular epithelium cell (podocyte). |
| 配列類似性 | Belongs to the podocalyxin family. |
| ドメイン | 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. |
| 翻訳後修飾 | N- and O-linked glycosylated. Sialoglycoprotein. |
| 細胞内局在 | 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 luminogenesis (By similarity).

製品の状態

There are 2 isoforms produced by alternative splicing.

アプリケーション

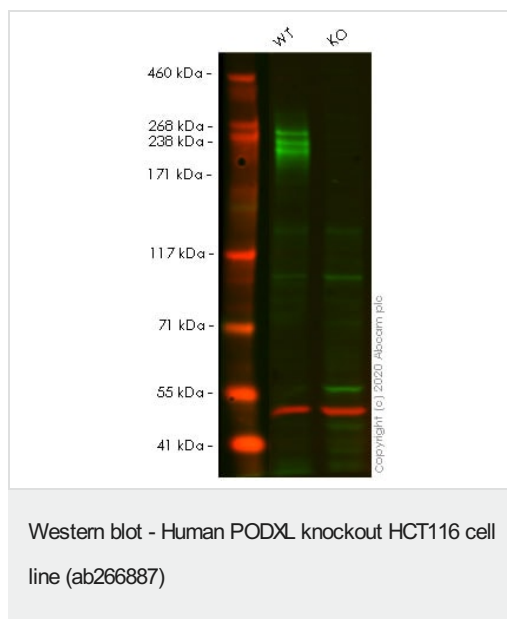
The Abpromise guarantee

Abpromise保証は、次のテスト済みアプリケーションにおけるab266887の使用に適用されませ

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: 58 kDa. |

画像



All lanes : Anti-PODXL antibody [EPR9518] (**ab150358**) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate

Lane 2 : PODXL knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

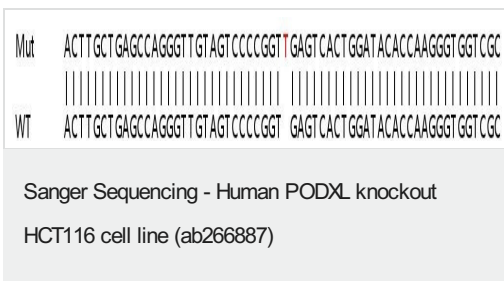
Performed under reducing conditions.

Predicted band size: 58 kDa

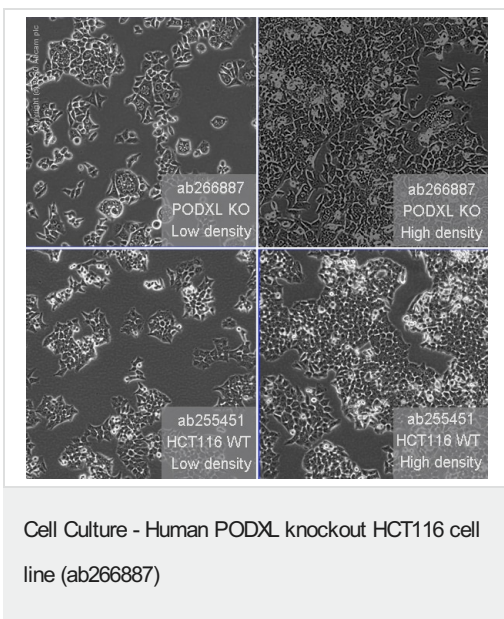
Observed band size: 200 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab150358** observed at 200 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab150358 was shown to 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 HCT116 and PODXL knockout HCT116 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. **ab150358** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 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.



Homozygous: 1 bp insertion in exon2



Representative images PODXL knockout HCT116 cells, low and high confluency examples (top left and right respectively) and wild-type HCT116 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS M5000 microscope.

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