abcam

Product datasheet

Human LRP6 knockout Hep G2 cell line ab277909

3 Images

Overview

Product name	Human LRP6 knockout Hep G2 cell line
Parental Cell Line	HepG2
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 46 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type Hep G2 cell line (**ab275467**). 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:** MEM + 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	Liver
Cell type	epithelial
Disease	Hepatocellular Carcinoma
Gender	Male
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	Component of the Wnt-Fzd-LRP5-LRP6 complex that triggers beta-catenin signaling through inducing aggregation of receptor-ligand complexes into ribosome-sized signalsomes. Cell-surface coreceptor of Wnt/beta-catenin signaling, which plays a pivotal role in bone formation. The Wnt-induced Fzd/LRP6 coreceptor complex recruits DVL1 polymers to the plasma membrane which, in turn, recruits the AXIN1/GSK3B-complex to the cell surface promoting the formation of signalsomes and inhibiting AXIN1/GSK3-mediated phosphorylation and destruction of beta-catenin. Required for posterior patterning of the epiblast during gastrulation.
Tissue specificity	Widely co-expressed with LRP5 during embryogenesis and in adult tissues.
Involvement in disease	Defects in LRP6 are the cause of autosomal dominant coronary artery disease type 2 (ADCAD2) [MIM:610947].
Sequence similarities	Belongs to the LDLR family. Contains 4 EGF-like domains. Contains 3 LDL-receptor class A domains. Contains 20 LDL-receptor class B repeats.
Domain	The YWTD-EGF-like domains 1 and 2 are required for the interaction with Wnt-frizzled complex. The YWTD-EGF-like domains 3 and 4 are required for the interaction with DKK1. The PPPSP motifs play a central role in signal transduction by being phosphorylated, leading to activate the Wnt signaling pathway.
Post-translational modifications	Dual phosphorylation of cytoplasmic PPPSP motifs sequentially by GSK3 and CK1 is required for AXIN1-binding, and subsequent stabilization and activation of beta-catenin via preventing GSK3-mediated phosphorylation of beta-catenin. Phosphorylated, in vitro, by GRK5/6 within and outside the PPPSP motifs. Phosphorylation at Ser-1490 by CDK14 during G2/M phase leads to regulation of the Wnt signaling pathway during the cell cycle. Phosphorylation by GSK3B is induced by RPSO1 binding and inhibited by DKK1. Phosphorylated, in vitro, by casein kinase I on Thr-1479.

	Undergoes gamma-secretase-dependent regulated intramembrane proteolysis (RIP). The extracellular domain is first released by shedding, and then, through the action of gamma-secretase, the intracellular domain (ICD) is released into the cytoplasm where it is free to bind to GSK3B and to activate canonical Wnt signaling. Palmitoylation on the two sites near the transmembrane domain leads to release of LRP6 from the endoplasmic reticulum. Mono-ubiquitinated which retains LRP6 in the endoplasmic reticulum. N-glycosylation is required for cell surface location.
Cellular localization	Membrane. Endoplasmic reticulum. On Wnt signaling, undergoes a cycle of caveolin- or clathrin-mediated endocytosis and plasma membrane location. Released from the endoplasmic reticulum on palmitoylation. Mono-ubiquitination retains it in the endoplasmic reticulum in the absence of palmitoylation. On Wnt signaling, phosphorylated, aggregates and colocalizes with AXIN1 and GSK3B at the plasma membrane in LRP6-signalsomes. Chaperoned to the plasma membrane by MESD.

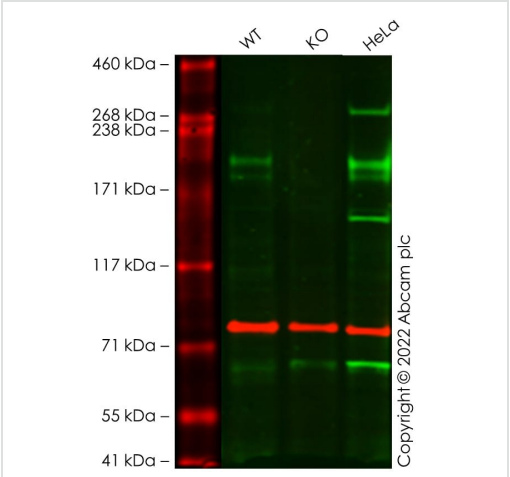
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab277909 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: 180 kDa.

Images



Western blot - Human LRP6 knockout Hep G2 cell line (ab277909)

All lanes : Anti-LRP6 antibody [EPR22910-39] (**ab231779**) at 1/1000 dilution

Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : LRP6 knockout HepG2 cell lysate
Lane 3 : HeLa cell lysate

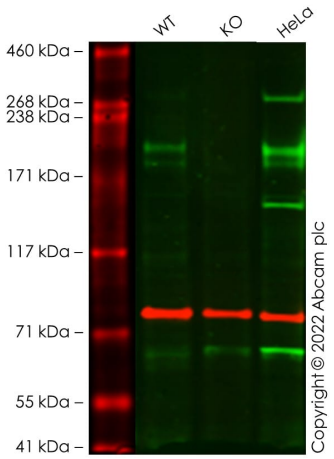
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 180 kDa
Observed band size: 180,200 kDa

False colour image of Western blot: Anti-LRP6 antibody [EPR22910-39] staining at 1/1000 dilution, shown in green; Mouse

anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab231779** was shown to bind specifically to LRP6. A band was observed at 180/200 kDa in wild-type HepG2 cell lysates with no signal observed at this size in LRP6 knockout cell line ab277909 (knockout cell lysate **ab284222**). To generate this image, wild-type and LRP6 knockout HepG2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



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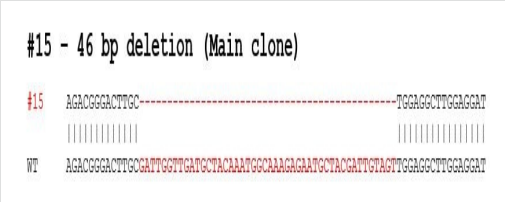

Sanger Sequencing - Human LRP6 knockout Hep G2 cell line (ab277909)

46 bp deletion in exon 2

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