

Human LRP5 knockout HEK-293T cell line ab266618

4 Images

Overview

Product name	Human LRP5 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 5 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HEK293T cell line (ab255449). 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: DMEM (High Glucose) + 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.</p> <p>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.

Properties

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
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	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. Appears to be required for postnatal control of vascular regression in the eye. Required for posterior patterning of the epiblast during gastrulation.
Tissue specificity	Widely expressed, with the highest level of expression in the liver.
Involvement in disease	<p>Defects in LRP5 are the cause of vitreoretinopathy exudative type 4 (EVR4) [MIM:601813]. EVR4 is a disorder of the retinal vasculature characterized by an abrupt cessation of growth of peripheral capillaries, leading to an avascular peripheral retina. This may lead to compensatory retinal neovascularization, which is thought to be induced by hypoxia from the initial avascular insult. New vessels are prone to leakage and rupture causing exudates and bleeding, followed by scarring, retinal detachment and blindness. Clinical features can be highly variable, even within the same family. Patients with mild forms of the disease are asymptomatic, and their only disease related abnormality is an arc of avascular retina in the extreme temporal periphery. EVR4 inheritance can be autosomal dominant or recessive.</p> <p>Genetic variations in LRP5 are a cause of susceptibility to osteoporosis (OSTEOP) [MIM:166710]; also known as senile osteoporosis or postmenopausal osteoporosis. Osteoporosis is characterized by reduced bone mass, disruption of bone microarchitecture without alteration in the composition of bone. Osteoporotic bones are more at risk of fracture.</p> <p>Defects in LRP5 are the cause of osteoporosis-pseudoglioma syndrome (OPPG) [MIM:259770]; also known as osteogenesis imperfecta ocular form. OPPG is a recessive disorder characterized</p>

by very low bone mass and blindness. Individually with OPPG are prone to develop bone fractures and deformations and have various eye abnormalities, including phthisis bulbi, retinal detachments, falciform folds or persistent vitreal vasculature.

Defects in LRP5 are a cause of high bone mass trait (HBM) [MIM:601884]. HBM is a rare phenotype characterized by exceptionally dense bones. HBM individuals show otherwise a completely normal skeletal structure and no other unusual clinical findings.

Defects in LRP5 are a cause of endosteal hyperostosis Worth type (WENHY) [MIM:144750]; also known as autosomal dominant osteosclerosis. WENHY is an autosomal dominant sclerosing bone dysplasia clinically characterized by elongation of the mandible, increased gonial angle, flattened forehead, and the presence of a slowly enlarging osseous prominence of the hard palate (torus palatinus). Serum calcium, phosphorus and alkaline phosphatase levels are normal. Radiologically, it is characterized by early thickening of the endosteum of long bones, the skull and of the mandible. With advancing age, the trabeculae of the metaphysis become thickened. WENHY becomes clinically and radiologically evident by adolescence, does not cause deformity except in the skull and mandible, and is not associated with bone pain or fracture. Affected patients have normal height, proportion, intelligence and longevity.

Defects in LRP5 are the cause of osteopetrosis autosomal dominant type 1 (OPTA1) [MIM:607634]. Osteopetrosis is a rare genetic disease characterized by abnormally dense bone, due to defective resorption of immature bone. The disorder occurs in two forms: a severe autosomal recessive form occurring in utero, infancy, or childhood, and a benign autosomal dominant form occurring in adolescence or adulthood. OPTA1 is characterized by generalized osteosclerosis most pronounced in the cranial vault. Patients are often asymptomatic, but some suffer from pain and hearing loss. It appears to be the only type of osteopetrosis not associated with an increased fracture rate.

Defects in LRP5 are the cause of van Buchem disease type 2 (VBCH2) [MIM:607636]. VBCH2 is an autosomal dominant sclerosing bone dysplasia characterized by cranial osteosclerosis, thickened calvaria and cortices of long bones, enlarged mandible and normal serum alkaline phosphatase levels.

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.

Post-translational modifications

Phosphorylation of cytoplasmic PPPSP motifs regulates the signal transduction of the Wnt signaling pathway through acting as a docking site for AXIN1.

Cellular localization

Membrane. Endoplasmic reticulum. Chaperoned to the plasma membrane by MESD.

Applications

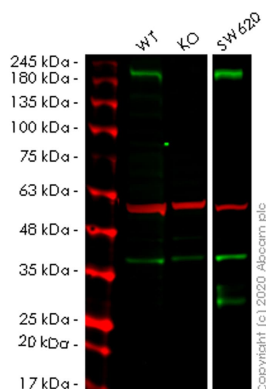
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab266618 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: 179 kDa.

Images



Western blot - Human LRP5 knockout HEK293T cell line (ab266618)

All lanes : Anti-LRP5 antibody [EPR22477-218] (**ab223203**) at 1/500 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : LRP5 knockout HEK293T cell lysate

Lane 3 : SW620 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 179 kDa

Observed band size: 180-200 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab223203** observed at 180-200 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab223203 Anti-LRP5 antibody [EPR22477-218] was shown to specifically react with LRP5 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266618 (knockout cell lysate **ab257202**) was used. Wild-type and LRP5 knockout samples were subjected to SDS-PAGE. **ab223203** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 500 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.

Mut	ACTCCGCCGGCGTCCACCAGCCGTACG-----GCGGTTGGCAATAGCAGGAGCGGCAG
WT	ACTCCGCCGGCGTCCACCAGCCGTACGTCGCGGCGTTGGCAATAGCAGGAGCGGCAG

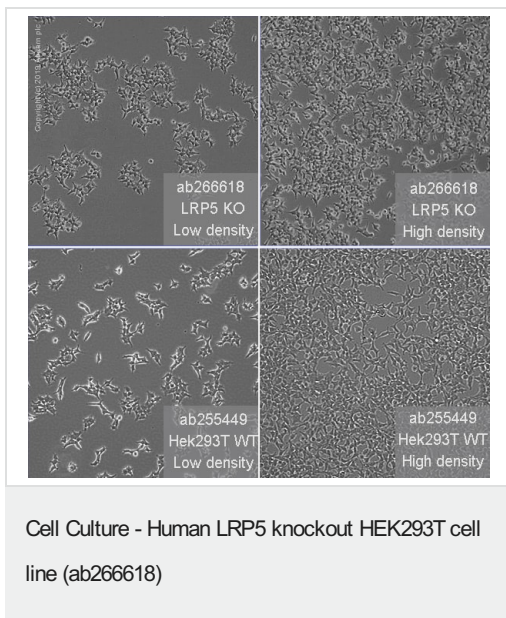
Sanger Sequencing - Human LRP5 knockout HEK293T cell line (ab266618)

Allele-1: 5 bp deletion in exon 2

Mut	ACTCCGCCGGCGTCCACCAGCGTACGGTCCCGGGCGTTGGCAAAATAGCAGGAGCGGCGA
WT	ACTCCGCCGGCGTCCACCAGCGTACG TCCCGGGCGTTGGCAAAATAGCAGGAGCGGCGA

Sanger Sequencing - Human LRP5 knockout
HEK293T cell line (ab266618)

Allele-2: 1 bp insertion in exon 2.



Representative images of LRP5 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T 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 XL Core microscope.

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