abcam

Product datasheet

Anti-LRP6 antibody [EPR22910-39] - BSA and Azide free ab256476

KO VALIDATED Recombinant RabMAb

3 Images

Overview

Product name	Anti-LRP6 antibody [EPR22910-39] - BSA and Azide free
Description	Rabbit monoclonal [EPR22910-39] to LRP6 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB **Unsuitable for:** Flow Cyt,ICC/IF,IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 (WT), HeLa, HepG2 and HEK-293T whole cell lysates.
General notes	ab256476 is the carrier-free version of **ab231779**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22910-39
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256476 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. Detects a band of approximately 180-210 kDa (predicted molecular weight: 180 kDa).

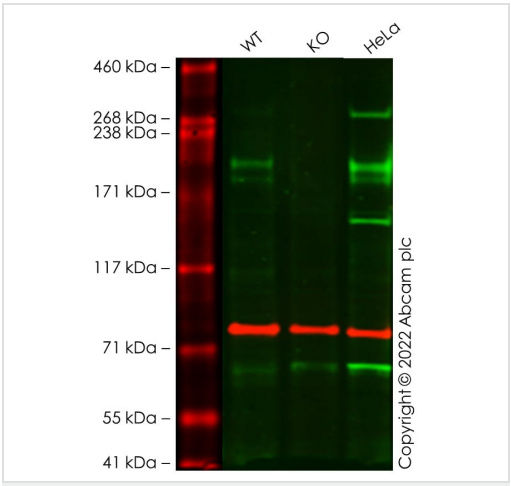
Application notes Is unsuitable for Flow Cyt,ICC/IF,IHC-P or IP.

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.

Images



Western blot - Anti-LRP6 antibody [EPR22910-39] - BSA and Azide free (ab256476)

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

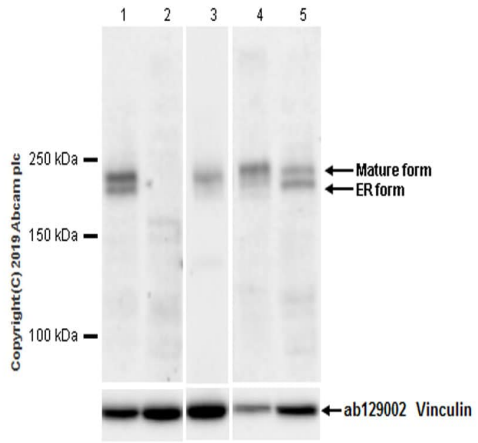
Lysates/proteins at 20 µg per lane.

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.



Western blot - Anti-LRP6 antibody [EPR22910-39] - BSA and Azide free (ab256476)

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

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : LRP6 knockout HAP1 whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 4 : HepG2 (human hepatocellular carcinoma epithelial cell), whole cell lysate
Lane 5 : 293T (human embryonic kidney epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 180 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab231779**).

The molecular weight observed is consistent with what has been described in the literature (PMID: 16989816, 16263759, 28341812). **ab231779** was shown to specifically react with LRP6 in wild-type HAP1 cells as signal was lost in LRP6 knockout cells. Wild-type and LRP6 knockout samples were subjected to SDS-PAGE. **ab231779** and **ab129002** (Rabbit anti-Vinculin loading control) were incubated 1 hour at room temperature at 1/1000

dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/20,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ instrument using the ECL technique. This blot was developed using a higher sensitivity ECL substrate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-2: 3 mins; Lanes 3: 26 secs; Lanes 4-5: 3 mins.

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