abcam

Product datasheet

Human LBR (Lamin B Receptor) knockout HEK-293T cell lysate ab257503

3 Images

Overview

Product name	Human LBR (Lamin B Receptor) knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
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)
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. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
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.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB, Sanger Sequencing

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260999 - Human LBR knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

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

Target

Function Anchors the lamina and the heterochromatin to the inner nuclear membrane.

Involvement in disease Defects in LBR are a cause of Pelger-Huet anomaly (PHA) [MIM:169400]. PHA is an autosomal dominant inherited abnormality of neutrophils, characterized by reduced nuclear segmentation and an apparently looser chromatin structure. Heterozygotes show hypolobulated neutrophil nuclei with coarse chromatin. Presumed homozygous individuals have ovoid neutrophil nuclei, as well as varying degrees of developmental delay, epilepsy, and skeletal abnormalities.
Defects in LBR are the cause of hydrops-ectopic calcification-moth-eaten skeletal dysplasia (HEM) [MIM:215140]; also known as Greenberg skeletal dysplasia. HEM is a rare autosomal recessive chondrodystrophy characterized by early in utero lethality and, therefore, considered to be nonviable. Affected fetuses typically present with fetal hydrops, short-limbed dwarfism, and a marked disorganization of chondro-osseous calcification and may present with polydactyly and additional nonskeletal malformations.
Defects in LBR may be a cause of Reynolds syndrome (REYNS) [MIM:613471]. It is a syndrome specifically associating limited cutaneous systemic sclerosis and primary biliray cirrhosis. It is characterized by liver disease, telangiectasia, abrupt onset of digital paleness or cyanosis in response to cold exposure or stress (Raynaud phenomenon), and variable features of scleroderma. The liver disease is characterized by pruritis, jaundice, hepatomegaly, increased serum alkaline phosphatase and positive serum mitochondrial autoantibodies, all consistent with primary biliary cirrhosis.

Sequence similarities Belongs to the ERG4/ERG24 family.

Post-translational modifications Phosphorylated by CDK1 protein kinase in mitosis when the inner nuclear membrane breaks down into vesicles that dissociate from the lamina and the chromatin. It is phosphorylated by different protein kinases in interphase when the membrane is associated with these structures. Phosphorylation of LBR and HP1 proteins may be responsible for some of the alterations in chromatin organization and nuclear structure which occur at various times during the cell cycle.

Cellular localization Nucleus inner membrane.

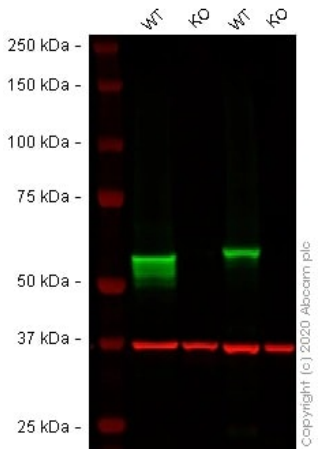
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257503 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: 71 kDa.
Sanger Sequencing		Use at an assay dependent concentration.

Images



Western blot - Human LBR knockout HEK293T cell lysate (ab257503)

Lane 1: Wild-type HEK-293T cell lysate (20 µg)

Lane 2: LBR knockout HEK-293T cell lysate (20 µg)

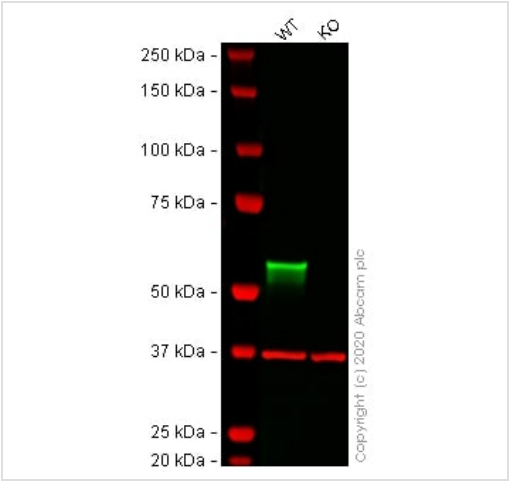
Lane 3: Wild-type MEF-1 whole cell lysate (20 µg)

Lane 4: LBR knockout MEF-1 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab232731** observed at 58 kDa. Red - loading control, **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

ab232731 was shown to react with Lamin B Receptor (LBR) in wild-type HEK-293 and MEF-1 cells in western blot. Loss of signal was observed when LBR knockout samples were used. Wild-type and LBR knockout (ab257503) HEK-293 cell lysates and wild-type and LBR knockout MEF-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk before incubation with **ab232731** and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

LBR knockout MEF-1 samples were kindly provided by the Brian Burke laboratory, A-Star Institute, Singapore.



Western blot - Human LBR knockout HEK293T cell lysate (ab257503)

Lane 1: Wild-type HEK-293T cell lysate (20 µg)

Lane 2: LBR knockout HEK-293T cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - **ab32535** observed at 58 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab32535 was shown to react with Lamin B Receptor/LBR in wild-type HEK-293 cells in western blot. Loss of signal was observed when LBR knockout lysate ab257503 was used. Wild-type and LBR knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk before incubation with **ab32535** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human LBR knockout HEK293T cell lysate (ab257503)

Homozygous: 1 bp insertion in exon 2

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