abcam

Product datasheet

Human NR2C2 (TR4) knockout HEK-293T cell lysate ab257563

3 Images

Overview

Product name	Human NR2C2 (TR4) knockout HEK-293T cell lysate
Product overview	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 16 bp deletion in exon6 and Insertion of the selection cassette in exon6.
Passage number	<20
Knockout validation	Sanger Sequencing
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

Properties

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

Components	1 kit
ab263568 - Human NR2C2 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 Orphan nuclear receptor that can act as a repressor or activator of transcription. An important repressor of nuclear recptor signaling pathways such as retinoic acid receptor, retinoid X, vitamin D3 receptor, thyroid hormone receptor and estrogen receptor pathways. May regulate gene expression during the late phase of spermatogenesis. Together with NR2C1, forms the core of the DRED (direct repeat erythroid-definitive) complex that represses embryonic and fetal globin transcription including that of GATA1. Binds to hormone response elements (HREs) consisting of two 5'-AGGTCA-3' half site direct repeat consensus sequences. Plays a fundamental role in early embryonic development and embryonic stem cells. Required for normal spermatogenesis and cerebellum development. Appears to be important for neurodevelopmentally regulated behavior (By similarity). Activates transcriptional activity of LHCG. Antagonist of PPARA-mediated transactivation.

Sequence similarities Belongs to the nuclear hormone receptor family. NR2 subfamily.
Contains 1 nuclear receptor DNA-binding domain.

Developmental stage Transiently repressed during the meiotic phase of spermatogenesis.

Post-translational modifications Phosphorylation on Ser-19 and Ser-68 is an important regulator of NR2C2-mediated transcriptional activity. Phosphorylation on these residues recruits the corepressor, NRIP1, leading to transcripional repression, whereas the nonphosphorylated form preferentially recruits the coactivator, PCAF.

Cellular localization Nucleus.

Form There are 2 isoforms produced by alternative splicing.

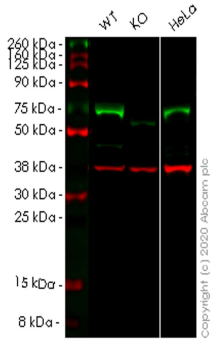
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257563 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: 65 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human NR2C2 (TR4) knockout HEK293T cell lysate (ab257563)

Lane 1: Wild-type HEK293T cell lysate (20 ug)

Lane 2: NR2C2 knockout HEK293T cell lysate (20 ug)

Lane 3: HeLa cell lysate (20 ug)

ab109301 Anti-TR4 antibody [EPR1773(2)] was shown to specifically react with TR4 in wild-type HEK293T cells. The band observed in knockout cell line **ab266228** (knockout cell lysate ab257563) lane below 67 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and TR4 knockout samples were subjected to SDS-PAGE. **ab109301** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours at 1 in 1000 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.

Sanger Sequencing - Human NR2C2 knockout HEK293T cell lysate (ab257563)

Allele-1: 16 bp deletion in exon6

Sanger Sequencing - Human NR2C2 knockout HEK293T cell lysate (ab257563)

Allele-2: Insertion of the selection cassette in exon6

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