abcam

Product datasheet

Human RXRA knockout HCT116 cell lysate ab275245

2 Images

Overview

Product name	Human RXRA knockout HCT116 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1
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

Properties

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

Components	1 kit
ab277288 - Human RXRA knockout HCT116 cell lysate	1 x 100µg
ab277289 - Human wild-type HCT116 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

Gender Male

Target

Function Receptor for retinoic acid. Retinoic acid receptors bind as heterodimers to their target response elements in response to their ligands, all-trans or 9-cis retinoic acid, and regulate gene expression in various biological processes. The RAR/RXR heterodimers bind to the retinoic acid response elements (RARE) composed of tandem 5'-AGGTCA-3' sites known as DR1-DR5. The high affinity ligand for RXRs is 9-cis retinoic acid. RXRA serves as a common heterodimeric partner for a number of nuclear receptors. The RXR/RAR heterodimers bind to the retinoic acid response elements (RARE) composed of tandem 5'-AGGTCA-3' sites known as DR1-DR5. In the absence of ligand, the RXR-RAR heterodimers associate with a multiprotein complex containing transcription corepressors that induce histone acetylation, chromatin condensation and transcriptional suppression. On ligand binding, the corepressors dissociate from the receptors and associate with the coactivators leading to transcriptional activation. The RXRA/PPARA heterodimer is required for PPARA transcriptional activity on fatty acid oxidation genes such as ACOX1 and the P450 system genes.

Tissue specificity Highly expressed in liver, also found in lung, kidney and heart.

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

Domain Composed of three domains: a modulating N-terminal domain (AF1 domain), a DNA-binding domain and a C-terminal ligand-binding domain (AF2 domain).

Post-translational modifications Phosphorylated on serine and threonine residues mainly in the N-terminal modulating domain. Constiutively phosphorylated on Ser-21 in the presence or absence of ligand. Under stress conditions, hyperphosphorylated by activated JNK on Ser-56, Ser-70, Thr-82 and Ser-260 (By similarity). Phosphorylated on Ser-27, in vitro, by PKA. This phosphorylation is required for repression of cAMP-mediated transcriptional activity of RARA.
Sumoylation negatively regulates transcriptional activity. Desumoylated specifically by SENP6.

Cellular localization Nucleus.

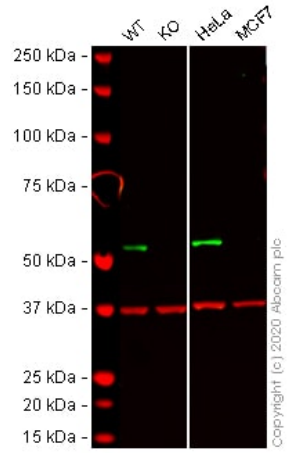
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275245 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

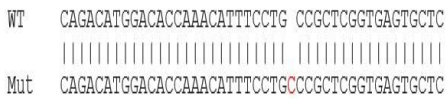
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 51 kDa.

Images



Western blot - Human RXRA knockout HCT116 cell lysate (ab275245)

Lane 1: Wild-type HCT116 cell lysate 20 ug
Lane 2: RXRA knockout HCT116 cell lysate 20 ug
Lane 3: HeLa cell lysate 20 ug
Lane 4: MCF7 cell lysate 20 ug
Lanes 1 - 4: Merged signal (red and green). Green - **ab125001** observed at 53 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab125001 was shown to react with Anti-Retinoid X Receptor alpha in wild-type HCT 116 cells in western blot with loss of signal observed in RXRA knockout cell line **ab273708** (RXRA knockout cell lysate ab275245). HCT 116 wild-type and RXRA knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab125001** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human RXRA knockout HCT116 cell lysate (ab275245)

Allele-1: 1 bp insertion in exon 1.

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