abcam

Product datasheet

Human RXRA knockout HCT116 cell line ab273708

3 Images

Overview

Product name	Human RXRA knockout HCT116 cell line
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	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC/IF
Biosafety level	1
General notes	**Recommended control:** Human wild-type HCT116 cell line (**ab273730**). 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** McCoY5a + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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	Colon
Cell type	epithelial
Disease	Carcinoma
Gender	Male
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	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.

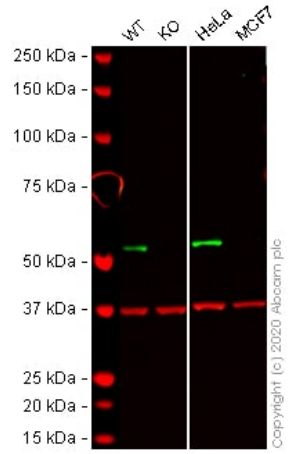
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273708 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.
ICC/IF		Use at an assay dependent concentration.

Images



Western blot - Human RXRA knockout HCT116 cell line (ab273708)

All lanes : Anti-Retinoid X Receptor alpha/RXRA antibody [EPR7106] (**ab125001**) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : RXRA knockout HCT116 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

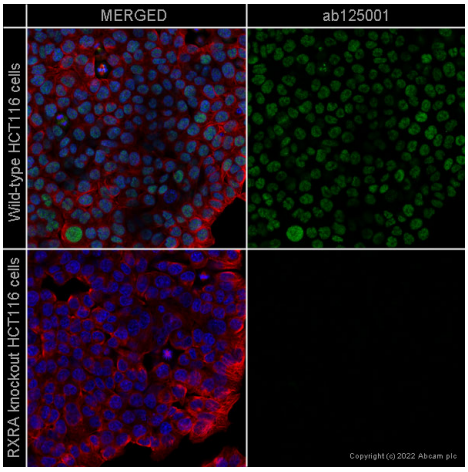
Performed under reducing conditions.

Predicted band size: 51 kDa
Observed band size: 53 kDa

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.

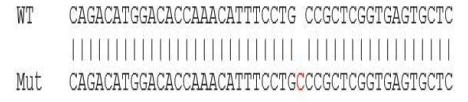
ab125001 was shown to react with Anti-Retinoid X Receptor alpha in wild-type HCT 116 cells in western blot with loss of signal observed in RXRA knockout cell line ab273708 (RXRA knockout cell lysate **ab275245**). Wild-type and RXRA knockout HCT 116 cell lysates were subjected to SDS-PAGE. Membranes 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.



Immunocytochemistry/ Immunofluorescence - Human RXRA knockout HCT116 cell line (ab273708)

ab125001 staining Retinoid X Receptor alpha in wild-type HCT116 cells (top panel) and RXRA knockout HCT116 cells (bottom panel) (ab273708). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab125001** at 0.2µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Sanger Sequencing - Human RXRA knockout HCT116 cell line (ab273708)

Allele-1: 1 bp insertion in exon 1.

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