

Human CFH (Factor H) knockout A549 cell line ab267031

5 Images

Overview

Product name	Human CFH (Factor H) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 10 bp deletion in exon 3 and 1 bp deletion in exon 3 and 2 bp deletion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type A549 cell line (ab255450). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: F-12K + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^3-1×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods.</p>

A guide seeding density of 6×10^4 cells/cm² 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.

Do not exceed 7×10^4 cells/cm².

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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	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 WWA: 14 TH01: 8,9,3 TPOX: 8,11 CSF1PO: 10, 12
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	Factor H functions as a cofactor in the inactivation of C3b by factor I and also increases the rate of dissociation of the C3bBb complex (C3 convertase) and the (C3b)NBB complex (C5 convertase) in the alternative complement pathway.
Tissue specificity	Expressed by the liver and secreted in plasma.
Involvement in disease	<p>Genetic variations in CFH are associated with basal laminar drusen (BLD) [MIM:126700]; also known as drusen of Bruch membrane or cuticular drusen or grouped early adult-onset drusen. Drusen are extracellular deposits that accumulate below the retinal pigment epithelium on Bruch membrane. Basal laminar drusen refers to an early adult-onset drusen phenotype that shows a pattern of uniform small, slightly raised yellow subretinal nodules randomly scattered in the macula. In later stages, these drusen often become more numerous, with clustered groups of drusen scattered throughout the retina. In time these small basal laminar drusen may expand and ultimately lead to a serous pigment epithelial detachment of the macula that may result in vision loss.</p> <p>Defects in CFH are the cause of complement factor H deficiency (CFH deficiency) [MIM:609814]. CFH deficiency determines uncontrolled activation of the alternative complement pathway with consumption of C3 and often other terminal complement components. It is associated with a number of renal diseases with variable clinical presentation and progression, including membranoproliferative glomerulonephritis and atypical hemolytic uremic syndrome. CFH</p>

deficiency patients may show increased susceptibility to meningococcal infections. Defects in CFH are a cause of susceptibility to hemolytic uremic syndrome atypical type 1 (AHUS1) [MIM:235400]. An atypical form of hemolytic uremic syndrome. It is a complex genetic disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure and absence of episodes of enterocolitis and diarrhea. In contrast to typical hemolytic uremic syndrome, atypical forms have a poorer prognosis, with higher death rates and frequent progression to end-stage renal disease. Note=Susceptibility to the development of atypical hemolytic uremic syndrome can be conferred by mutations in various components of or regulatory factors in the complement cascade system. Other genes may play a role in modifying the phenotype.

Genetic variation in CFH is associated with age-related macular degeneration type 4 (ARMD4) [MIM:610698]. ARMD is a multifactorial eye disease and the most common cause of irreversible vision loss in the developed world. In most patients, the disease is manifest as ophthalmoscopically visible yellowish accumulations of protein and lipid (known as drusen) that lie beneath the retinal pigment epithelium and within an elastin-containing structure known as Bruch membrane.

Sequence similarities

Contains 20 Sushi (CCP/SCR) domains.

Cellular localization

Secreted.

Applications

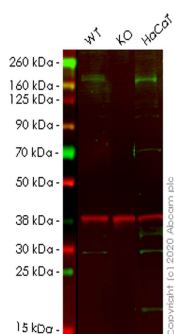
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab267031 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: 139 kDa.

Images



Western blot - Human CFH (Factor H) knockout A549 cell line (ab267031)

All lanes : Anti-Factor H antibody [EPR6225] (**ab133536**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : CFH knockout A549 cell lysate

Lane 3 : HaCaT cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 139 kDa

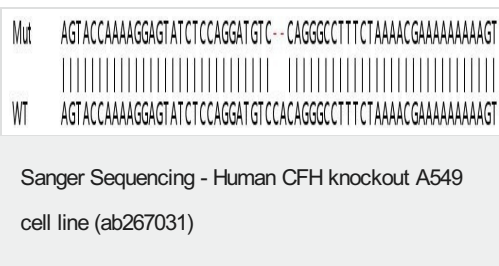
Observed band size: 180 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab133536** observed at 180 kDa. Red - loading control **ab8245** observed at 36 kDa.

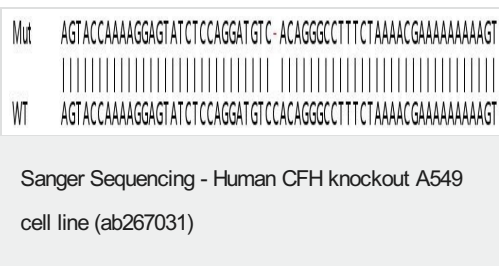
ab133536 Anti-Factor H antibody [EPR6225] was shown to specifically react with Factor H in wild-type A549 cells. Loss of signal was observed when knockout cell line ab267031 (knockout cell lysate **ab257150**) was used. Wild-type and Factor H knockout samples were subjected to SDS-PAGE. **ab133536** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C 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.



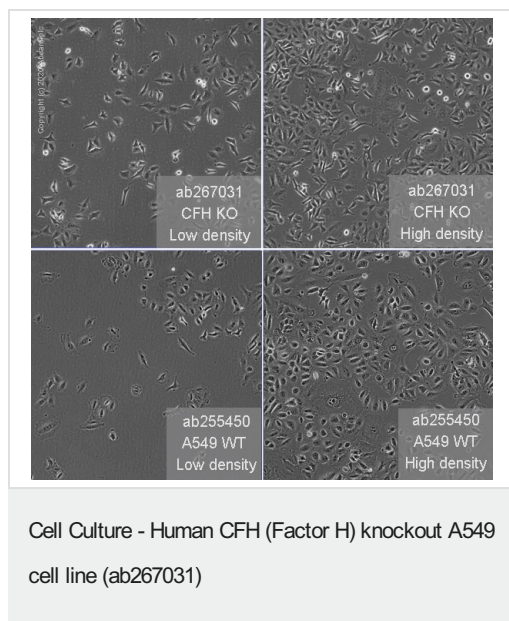
Allele-1: 10 bp deletion in exon3



Allele-2: 2 bp deletion in exon 3.



Allele-3: 1 bp deletion in exon 3.



Representative images of CFH knockout A549 cells, low and high confluency examples (top left and right respectively) and wild-type A549 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

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