abcam

Product datasheet

Human AES (Amino-terminal enhancer of split) knockout HCT116 cell line ab266886

3 Images

Overview

Product name	Human AES (Amino-terminal enhancer of split) knockout HCT116 cell line
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type HCT116 cell line (ab255451). 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 -80ºC. Storage at -80ºC may result in loss of viability. 1. Thaw the vial in 37ºC water bath 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 (Click here to view haemocytometer protocol) 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. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37ºC 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 for confluency (80-90% confluence) within 48 hours.
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.
Click here to view the Mammalian cell tissue culture protocol

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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~90%
Adherent /Suspension	Adherent
Tissue	Colon
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X D5S818: 10, 11 D13S317: 10, 12 D7S820: 11, 12 D16S539: 11, 13 vWA: 17, 22 TH01: 8,9 TPOX: 8, 9 CSF1PO: 7, 10
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether
Purity	Immunogen affinity purified

Target

Function	Transcriptional corepressor. Acts as dominant repressor towards other family members. Inhibits NF-kappa-B-regulated gene expression. May be required for the initiation and maintenance of the differentiated state. Essential for the transcriptional repressor activity of SIX3 during retina and lens development.
Tissue specificity	Found predominantly in muscle, heart and Placenta. In fetal tissues, abundantly expressed in the heart, lung, kidney, brain and liver.
Sequence similarities	Belongs to the WD repeat Groucho/TLE family.
Domain	Lacks the C-terminal WD repeats.
Post-translational modifications	Ubiquitinated by XIAP/BIRC4.
Cellular localization	Nucleus.

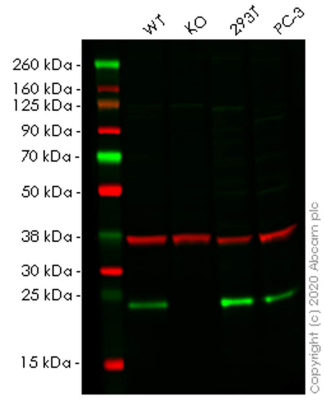
Applications

Our Abpromise guarantee covers the use of **ab266886** 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: 21 kDa.

Images



Western blot - Human AES (Amino-terminal enhancer of split) knockout HCT116 cell line (ab266886)

All lanes : Anti-Amino-terminal enhancer of split/AES antibody [EPR8385] (ab137060) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 (Human colorectal carcinoma cell line) whole cell lysate
Lane 2 : AES knockout HCT 116 (Human colorectal carcinoma cell line) whole cell lysate
Lane 3 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 4 : PC3 (Human prostate adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

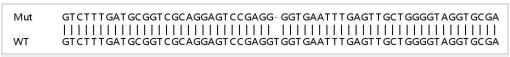
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 21 kDa
Observed band size: 21 kDa

Lanes 1-4: Merged signal (red and green). Green - ab137060 observed at 21 kDa. Red - loading control ab8245 observed at 36 kDa.

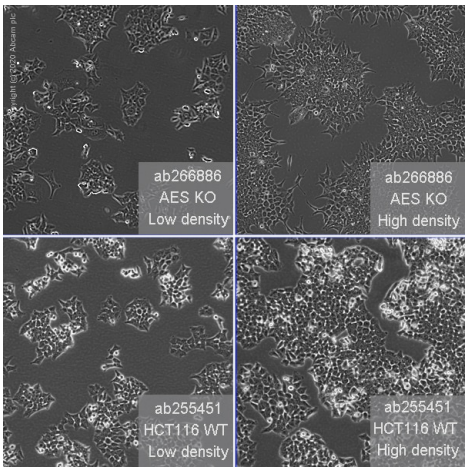
ab137060 Anti-Amino-terminal enhancer of split/AES antibody [EPR8385] was shown to specifically react with Amino-terminal enhancer of split/AES in wild-type HCT cells. Loss of signal was observed when knockout cell line ab266886 (knockout cell lysate ab257818) was used. Wild-type and Amino-terminal enhancer of split/AES knockout samples were subjected to SDS-PAGE. ab137060 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.



Sanger Sequencing - Human AES knockout HCT116 cell line (ab266886)

Homozygous: 1 bp deletion in exon2



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