abcam

Product datasheet

Human TFAP2A (Transcription factor AP-2-alpha) knockout HeLa cell line ab265122

3 Images

Overview

Product name	Human TFAP2A (Transcription factor AP-2-alpha) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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:** DMEM (High Glucose) + 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	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
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	Sequence-specific DNA-binding protein that interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes. AP-2 factors bind to the consensus sequence 5'-GCCNNNGGC-3' and activate genes involved in a large spectrum of important biological functions including proper eye, face, body wall, limb and neural tube development. They also suppress a number of genes including MCAM/MUC18, C/EBP alpha and MYC. AP-2-alpha is the only AP-2 protein required for early morphogenesis of the lens vesicle.
Involvement in disease	Defects in TFAP2A are the cause of branchiooculofacial syndrome (BOFS) [MIM:113620]; also known as branchial clefts with characteristic facies, growth retardation, imperforate nasolacrimal duct, and premature aging or lip pseudocleft-hemangiomatous branchial cyst syndrome. BOFS is a rare autosomal dominant cleft palate craniofacial disorder with variable expressivity. The major features include cutaneous anomalies, ocular anomalies, characteristic facial appearance (malformed pinnae, oral clefts), and, less commonly, renal and ectodermal (dental and hair) anomalies.
Sequence similarities	Belongs to the AP-2 family.
Domain	The WW-binding motif mediates interaction with WWOX.
Post-translational modifications	Sumoylated on Lys-10; which inhibits transcriptional activity.
Cellular localization	Nucleus.

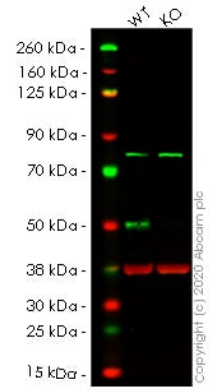
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265122 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: 48 kDa.

Images



Western blot - Human TFAP2A knockout HeLa cell line (ab265122)

All lanes : Anti-Transcription factor AP-2-alpha antibody [EPR2688(2)] (**ab108311**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : TFAP2A knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 48 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab108311** observed at 48 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab108311 was shown to react with Transcription factor AP-2-alpha in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265122 (knockout cell lysate **ab257736**) was used. Wild-type HeLa and TFAP2A knockout HeLa cell lysates were subjected to SDS-PAGE. **ab108311** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 Dilution and a 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 TFAP2A knockout HeLa cell line (ab265122)

Allele-1: 1 bp insertion in exon 2.

Sanger Sequencing - Human TFAP2A knockout HeLa cell line (ab265122)

Allele-2: Insertion of the selection cassette in exon 2.

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