abcam

Product datasheet

Human BAX knockout HeLa cell line ab255363

4 Images

Overview

Product name	Human BAX knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion 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 (**ab255448**). 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	Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19k protein. Under stress conditions, undergoes a conformation change that causes translocation to the mitochondrion membrane, leading to the release of cytochrome c that then triggers apoptosis. Promotes activation of CASP3, and thereby apoptosis.
Tissue specificity	Expressed in a wide variety of tissues. Isoform Psi is found in glial tumors. Isoform Alpha is expressed in spleen, breast, ovary, testis, colon and brain, and at low levels in skin and lung. Isoform Sigma is expressed in spleen, breast, ovary, testis, lung, colon, brain and at low levels in skin. Isoform Alpha and isoform Sigma are expressed in pro-myelocytic leukemia, histiocytic lymphoma, Burkitt's lymphoma, T-cell lymphoma, lymphoblastic leukemia, breast adenocarcinoma, ovary adenocarcinoma, prostate carcinoma, prostate adenocarcinoma, lung carcinoma, epidermoid carcinoma, small cell lung carcinoma and colon adenocarcinoma cell lines.
Sequence similarities	Belongs to the Bcl-2 family.
Domain	Intact BH3 motif is required by BIK, BID, BAK, BAD and BAX for their pro-apoptotic activity and for their interaction with anti-apoptotic members of the Bcl-2 family.
Cellular localization	Cytoplasm and Mitochondrion membrane. Cytoplasm. Colocalizes with 14-3-3 proteins in the cytoplasm. Under stress conditions, undergoes a conformation change that causes release from JNK-phosphorylated 14-3-3 proteins and translocation to the mitochondrion membrane.

Applications

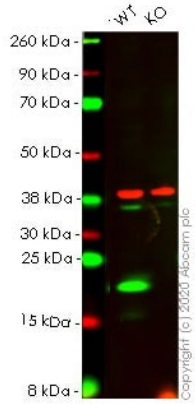
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255363 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 BAX knockout HeLa cell line (ab255363)

All lanes : Anti-Bax antibody [EPR18284] (**ab182734**) at 1/1000 dilution

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

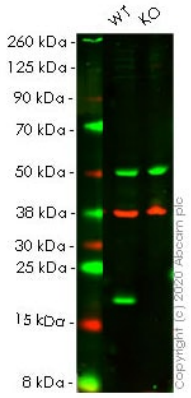
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1- 2: Merged signal (red and green). Green - **ab182734** observed at 21 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab182734 was shown to react with BAX in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255363 (knockout cell lysate **ab263841**) was used. Wild-type HeLa and BAX knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab182734** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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.



Western blot - Human BAX knockout HeLa cell line (ab255363)

All lanes : Anti-Bax antibody [E63] (**ab32503**) at 1/1000 dilution

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

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

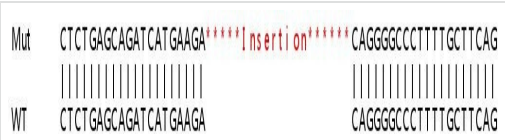
Predicted band size: 21 kDa
Observed band size: 21 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab32503** observed at 21 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32503 was shown to react with BAX in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255363 (knockout cell lysate **ab263841**) was used. Wild-type HeLa and BAX knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab32503** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 BAX knockout HeLa cell line (ab255363)

Allele-1: 1 bp deletion in exon 2.



Sanger Sequencing - Human BAX knockout HeLa cell line (ab255363)

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

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