

Product datasheet

Human BAD knockout HeLa cell line ab264843

3 Images

Overview

Product name	Human BAD knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 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	<p>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.</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: DMEM (High Glucose) + 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 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 2x10⁴ cells/cm². 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 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. A guide seeding density of 2x10⁴ cells/cm² is recommended for confluency (80-90% confluence)</p>

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	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 wWA: 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% DMSO, 2% Cellulose, methyl ether

Target

Function	Promotes cell death. Successfully competes for the binding to Bcl-X(L), Bcl-2 and Bcl-W, thereby affecting the level of heterodimerization of these proteins with BAX. Can reverse the death repressor activity of Bcl-X(L), but not that of Bcl-2 (By similarity). Appears to act as a link between growth factor receptor signaling and the apoptotic pathways.
Tissue specificity	Expressed in a wide variety of tissues.
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.
Post-translational modifications	Phosphorylated on one or more of Ser-75, Ser-99, Ser-118 and Ser-134 in response to survival stimuli, which blocks its pro-apoptotic activity. Phosphorylation on Ser-99 or Ser-75 promotes heterodimerization with 14-3-3 proteins. This interaction then facilitates the phosphorylation at Ser-118, a site within the BH3 motif, leading to the release of Bcl-X(L) and the promotion of cell survival. Ser-99 is the major site of AKT/PKB phosphorylation, Ser-118 the major site of protein kinase A (CAPK) phosphorylation. Ser-75 is phosphorylated by AKT/PKB, protein kinase A and PIM2.
Cellular localization	Mitochondrion outer membrane. Cytoplasm. Upon phosphorylation, locates to the cytoplasm.

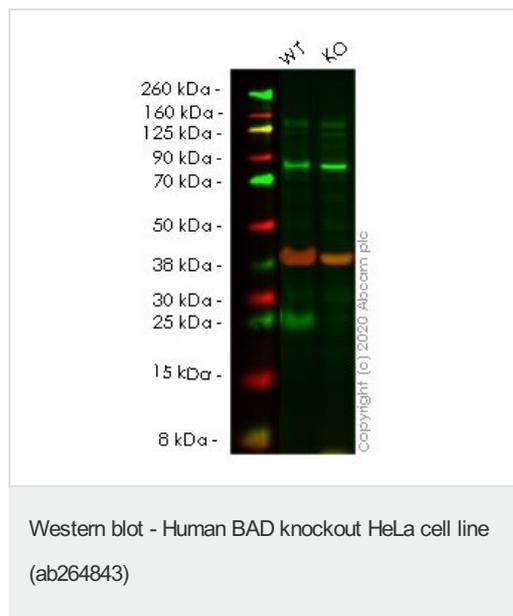
Applications

Our [Abpromise guarantee](#) covers the use of **ab264843** 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: 18 kDa.

Images



All lanes : Anti-Bad antibody [Y208] ([ab32445](#)) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : BAD knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 18 kDa

Observed band size: 23 kDa

[why is the actual band size different from the predicted?](#)

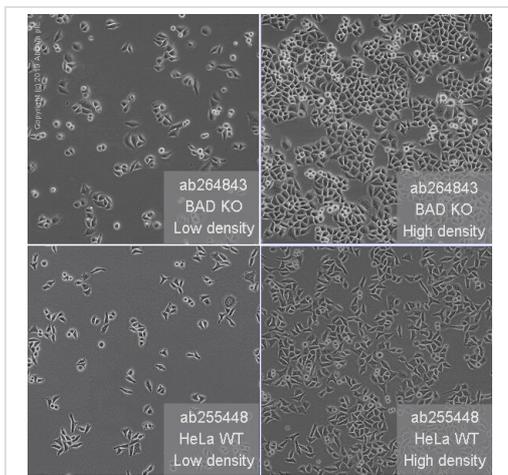
Lanes 1- 2: Merged signal (red and green). Green - [ab32445](#) observed at 23 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab32445](#) was shown to react with BAD in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line [ab264843](#) (knockout cell lysate [ab256847](#)) was used. Wild-type HeLa and BAD 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. [ab32445](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 2000 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.

Mut	TGGT GACTGGCGTCCCACAG*****[insertion]*****GAGGCCTGGGCCTGGCGAT
WT	TGGT GACTGGCGTCCCACAGGAGGCCTGGGCCTGGCGAT

Homozygous: Insertion of the selection cassette in exon 2.

Sanger Sequencing - Human BAD knockout HeLa cell line (ab264843)



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

Cell Culture - Human BAD knockout HeLa cell line (ab264843)

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