

Product datasheet

Human BAK1 (Bak) knockout HeLa cell lysate ab257077

5 Images

Overview

Product name	Human BAK1 (Bak) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon2 and 2 bp deletion in exon2 and Insertion of the selection cassette in exon2.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab261957 - Human BAK1 knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

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

Target

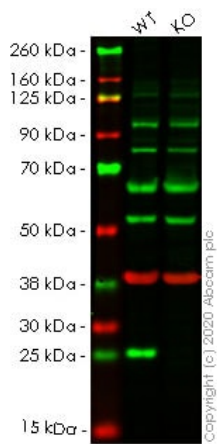
Function	In the presence of an appropriate stimulus, accelerates programmed cell death by binding to, and antagonizing the anti-apoptotic action of BCL2 or its adenovirus homolog E1B 19k protein. Low micromolar levels of zinc ions inhibit the promotion of apoptosis.
Tissue specificity	Expressed in a wide variety of tissues, with highest levels in the heart and skeletal muscle.
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	Mitochondrion membrane.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257077 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: 23 kDa.

Images



Western blot - Human BAK1 (Bak) knockout HeLa cell lysate (ab257077)

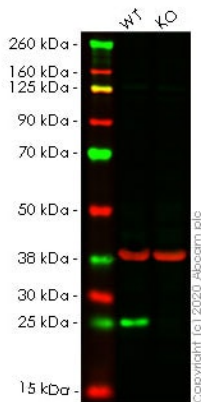
Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: BAK1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab92999** observed at 23 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab92999 Anti-Bak antibody was shown to specifically react with Bak in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265277** (knockout cell lysate ab257077) was used. Wild-type and Bak knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk.

ab92999 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4 °C at 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 BAK1 (Bak) knockout HeLa cell lysate (ab257077)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: BAK1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab32371** observed at 23 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab32371 Anti-Bak antibody [Y164] was shown to specifically react with Bak in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265277** (knockout cell lysate ab257077) was used. Wild-type and Bak knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk.

ab32371 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.

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Mut  CAGGCAGGAGTGGGAGAGCCTGCCCTGCC--CTGCTTCTGGTAAGGGTCTTCCTGCCCC
      |||
WT   CAGGCAGGAGTGGGAGAGCCTGCCCTGCCCTGCTTCTGGTAAGGGTCTTCCTGCCCC

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Sanger Sequencing - Human BAK1 knockout HeLa
cell lysate (ab257077)

Allele-1: 2 bp deletion in exon2

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Mut  CAGGCAGGAGTGGGAGAGCCTGCCCTGCC-TCTGCTTCTGGTAAGGGTCTTCCTGCCCC
      |||
WT   CAGGCAGGAGTGGGAGAGCCTGCCCTGCCCTGCTTCTGGTAAGGGTCTTCCTGCCCC

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Sanger Sequencing - Human BAK1 knockout HeLa
cell lysate (ab257077)

Allele-2: 1 bp deletion in exon2

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Mut  CTCCCAGGCAGGAGTGGGGA****Insertion*****GAGCCTGCCCTGCCCTCTGC
      |||
WT   CTCCCAGGCAGGAGTGGGGA                GAGCCTGCCCTGCCCTCTGC

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Sanger Sequencing - Human BAK1 knockout HeLa
cell lysate (ab257077)

Allele-3: Insertion of the selection cassette in exon2

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