abcam

ab32371

Anti-Bak antibody [Y164]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y164
Purification technique	Affinity purification Protein A
Specificity	This antibody recognises Bak. The antibody does not cross-react with other Bcl2 members.
Concentration	0.612 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	1/10000
Notes	**For unpurified use at 1/1000 - 1/5000.**

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	**For unpurified use at 1/250-1/500.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/20
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information BAK1

Function	Plays a role in the mitochondrial apoptotic process. Upon arrival of cell death signals, promotes mitochondrial outer membrane (MOM) permeabilization by oligomerizing to form pores within the MOM. This releases apoptogenic factors into the cytosol, including cytochrome c, promoting the activation of caspase 9 which in turn processes and activates the effector caspases.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Bak protein also known as Bcl-2 homologous antagonist killer plays a mechanical role in the mitochondrial apoptosis pathway. It often functions as a pro-apoptotic member of the Bcl-2 protein family. Bak is expressed widely in the body being present in tissues such as the brain and heart. The molecular weight of Bak is approximately 23 kilodaltons. Bak interacts with other mitochondrial proteins to trigger cell death processes.
Biological function summary	Bak engages in promoting apoptosis by disrupting the mitochondrial outer membrane potential. It is part of the Bcl-2 family complex which balances cell survival and cell death. Bak collaborates with Bax protein to form pores in the mitochondrial membrane releasing cytochrome c and other apoptotic factors. This process initiates the cascade of caspases leading to programmed cell death.
Pathways	Bak is a critical component of the intrinsic apoptotic pathway. It directly interacts with pro-apoptotic proteins like Bax and anti-apoptotic proteins such as Bcl-2 and Bcl-xL. Bak's action in the apoptotic pathway involves a delicate balance between survival and death signals affecting cellular homeostasis. In addition Bak's interaction with the p53 pathway emphasizes its role in response to DNA damage ensuring damaged cells do not proliferate uncontrollably.
Associated diseases and disorders	Malfunction or dysregulation of Bak can lead to cancer and neurodegenerative disorders. In cancer reduced Bak activity may allow cells to evade apoptosis promoting tumor survival and growth. Conversely in diseases like Parkinson's excessive Bak activity may result in enhanced neuronal apoptosis. Bak's interaction with proteins such as Bcl-2 also makes it a potential target for therapies aimed at restoring apoptotic balance in affected cells.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

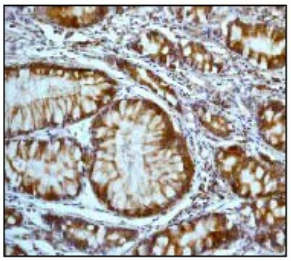
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

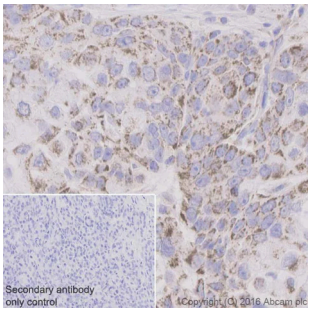
14 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

Bak Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bak antibody

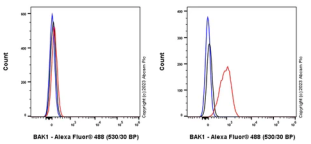
Immunohistochemical analysis of Bak expression in paraffin embedded human stomach carcinoma, using 1/250 unpurified ab32371.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

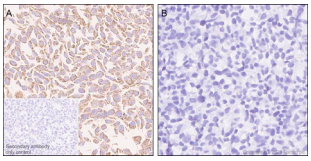
Bak Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bak antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human pancreatic carcinoma tissue sections labeling Bak with Purified ab32371 at 1:200 dilution (2.98 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



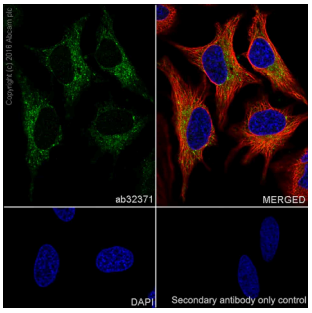
Flow Cytometry (Intracellular) - Anti-Bak antibody [Y164] (ab32371)

Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized BAK1 KO Hela (human BAK1 knockout cervical adenocarcinoma epithelial cell, Left) /Parental Hela (Right) cells labelling Bak with ab32371 at 1/500 dilution (0.1 µg)(Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

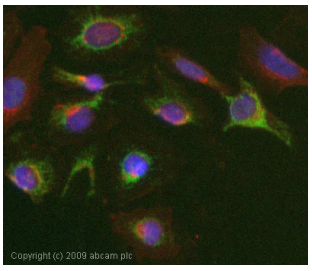
Immunohistochemical analysis of paraffin-embedded fixed (A) Wild-type HeLa (human cervix adenocarcinoma epithelial cell) cell pellet. (B) BAK1 knockout HeLa (ab265277) cell pellet staining Bak with ab32371 at 1/2000 dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Counter-staining used was hematoxylin. Positive staining on (A) Wild-type HeLa cell pellet, no staining on (B) BAK1 knockout HeLa (ab265277) cell pellet. The section was incubated with ab32371 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Bak antibody [Y164] (ab32371)

Bak Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bak antibody

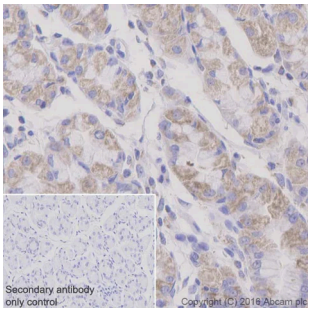
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Bak with Purified ab32371 at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200. ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunocytochemistry/ Immunofluorescence - Anti-Bak antibody [Y164] (ab32371)

Bak Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Bak antibody

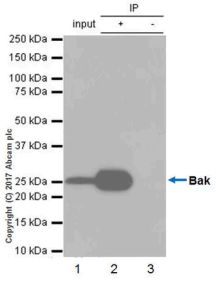
ICC/IF image of unpurified ab32371 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab32371, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] (ab32371)

Bak Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Bak antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach tissue sections labeling Bak with Purified ab32371 at 1:200 dilution (2.98 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

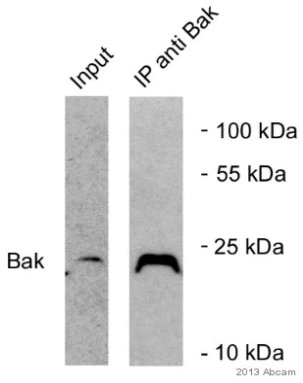
ab32371 (purified) at 1:20 dilution (2µg) immunoprecipitating Bak in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32371 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32371 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.
All lanes:

Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

Predicted band size: 23 kDa

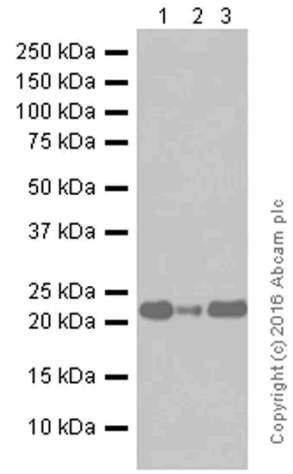


Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

Bak was immunoprecipitated from HCT116 p53-/- cell line whole cell lysate with unpurified ab32371 at 1/100 dilution.
Western blot was performed from the immunoprecipitate using ab32371 at 1/2000 dilution.

All lanes:
Immunoprecipitation - Anti-Bak antibody [Y164] (ab32371)

Predicted band size: 23 kDa



Western blot - Anti-Bak antibody [Y164] (ab32371)

Bak Western blot staining using rabbit Anti-Bak antibody

Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Bak antibody [Y164] (ab32371) at 1/10000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Lane 2:
HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates at 20 µg

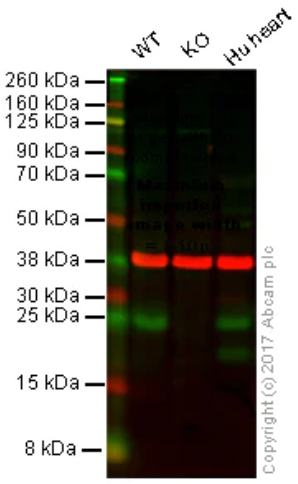
Lane 3:
Human fetal heart lysates at 20 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 23 kDa

Observed band size: 23 kDa



Western blot - Anti-Bak antibody [Y164] (ab32371)

Lanes 1 - 3: Merged signal (red and green). Green - ab32371 observed at 25 kDa. Red - loading control, ab9484, observed at 37 kDa.
Unpurified ab32371 was shown to specifically recognize BAK in wild-type HAP1 cells. No band was observed when BAK knockout cells were examined. Wild-type and BAK knockout samples were subjected to SDS-PAGE. Unpurified ab32371 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.

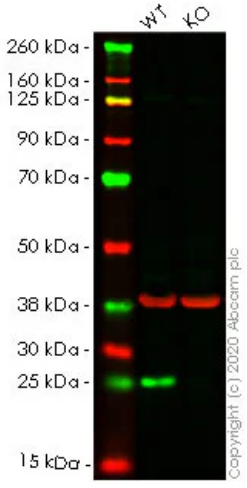
All lanes:
Western blot - Anti-Bak antibody [Y164] (ab32371)

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
BAK knockout HAP1 whole cell lysate at 20 µg

Lane 3:
Human Heart whole cell lysate at 20 µg

Predicted band size: 23 kDa



Western blot - Anti-Bak antibody [Y164] (ab32371)

Bak Western blot staining using rabbit Anti-Bak antibody

Lanes 1- 2: Merged signal (red and green). Green - ab32371 observed at 23 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.
ab32371 was shown to 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 HeLa and BAK1 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. ab32371 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.

All lanes:
Western blot - Anti-Bak antibody [Y164] (ab32371) at 1/1000 dilution

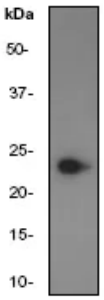
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human BAK1 (Bak) knockout HeLa cell lysate (ab257077) at 20 µg

Performed under reducing conditions.

Predicted band size: 23 kDa

Observed band size: 23 kDa



Western blot - Anti-Bak antibody [Y164] (ab32371)

All lanes:
Western blot - Anti-Bak antibody [Y164] (ab32371) at 1/5000 dilution

All lanes:
HeLa cell lysate

Predicted band size: 23 kDa

Observed band size: 23 kDa

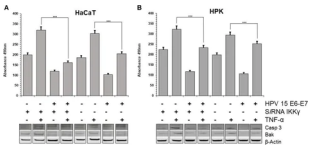


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Western blot - Anti-Bak antibody [Y164] (ab32371)

Bak Western blot staining using rabbit Anti-Bak antibody

Bak western blot using anti-Bak antibody [Y164] ab32371. Publication image and figure legend from Paolini, F., Zaccarini, M., et al., 2020, Front Cell Infect Microbiol, PubMed 32257968.

ab32371 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab32371 please see the product overview.

HPV15 affects TNF-α-induced apoptosis. HaCaT (A) and HPK (B) cells were utlized. HPV 15 transduction, IKKγ gene silencing and TNF-α treatment (10 ng/ml) for 6 h are indicated. Apoptosis was measured by a Cell Death ELISA test according to manufacturer's instruction in triplicate samples. Error bars = ±SD. ***p < 0.0001 by unpaired t-test. Activation of apoptotic pathway was analyzed in cell lysates by Western blots with anti-cleaved Caspase 3 or anti-BAK antibodies, as described in section Materials and Methods. All these experiments were repeated twice with similar results.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.