abcam

Product datasheet

Anti-Bak antibody [Y164] - BSA and Azide free ab220790

KO VALIDATED Recombinant RabMAb

21 References 10 Images

Overview

Product name	Anti-Bak antibody [Y164] - BSA and Azide free
Description	Rabbit monoclonal [Y164] to Bak - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises Bak. The antibody does not cross-react with other Bcl2 members.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Hap1, human heart cells. IP: HeLa and HCT116 cells. Flow Cyt (intra): HeLa cells. IHC-P: Human pancreatic carcinoma, stomach carcinoma and stomach tissue.
General notes	ab220790 is the carrier-free version of **ab32371**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y164
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220790 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

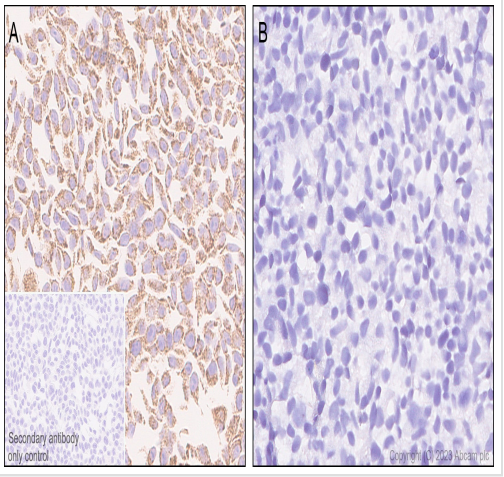
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

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.

Images

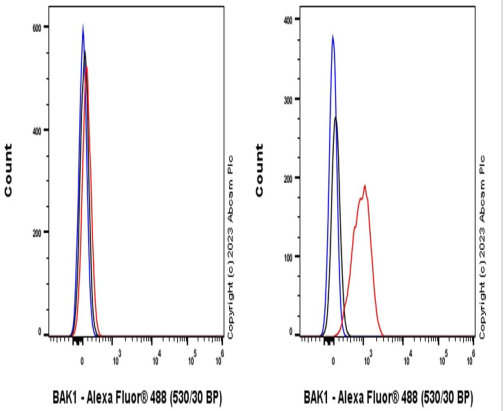


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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

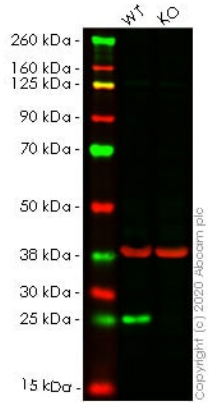
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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.



Western blot - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BAK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

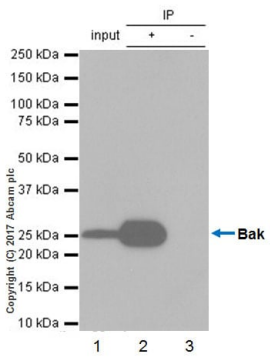
Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 23 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab32371**).

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.



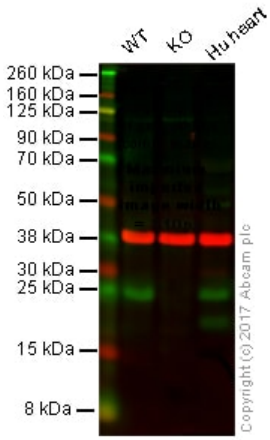
Immunoprecipitation - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32371**).



Western blot - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : BAK knockout HAP1 whole cell lysate
Lane 3 : Human Heart whole cell lysate

Lysates/proteins at 20 µg per lane.

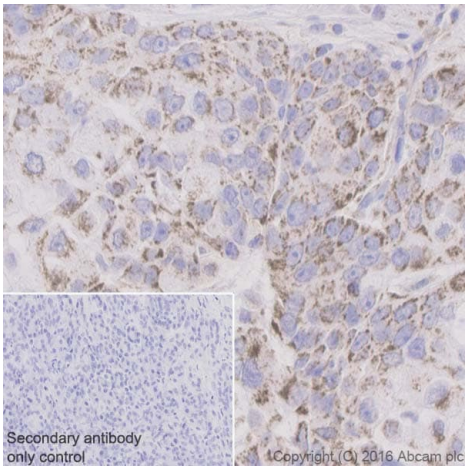
Predicted band size: 23 kDa

This WB data was generated using the same anti-Bak antibody clone, Y164, in a different buffer formulation (cat# **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)

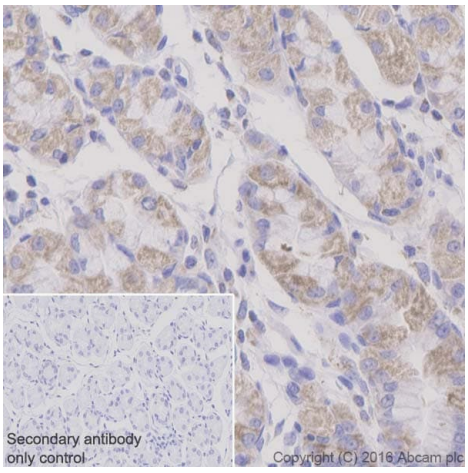
preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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.

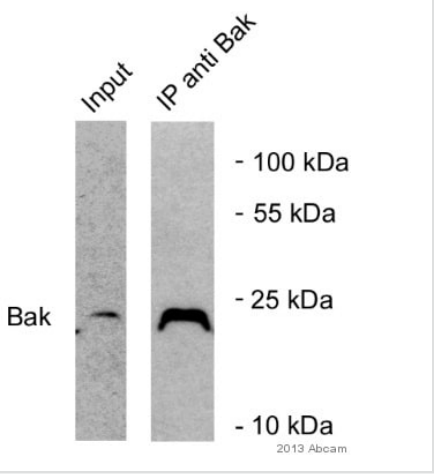
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32371**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32371**).

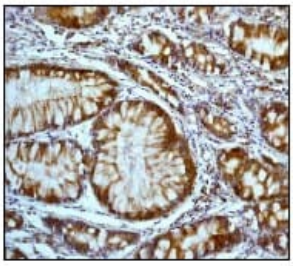


Immunoprecipitation - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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.

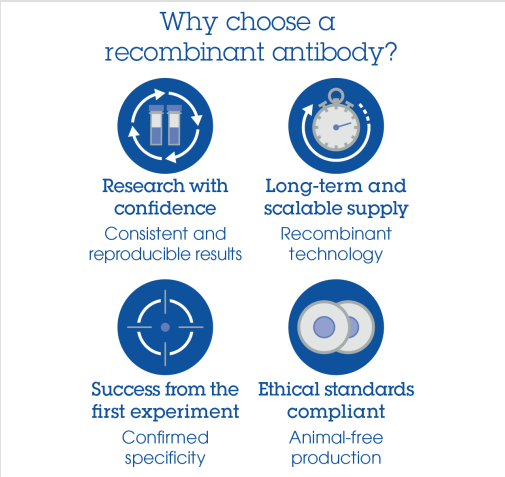
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32371**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bak antibody [Y164] - BSA and Azide free (ab220790)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32371**).



Anti-Bak antibody [Y164] - BSA and Azide free

(ab220790)

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