

Anti-BAK/BAK1 Antibody Picoband®

Catalog Number: PB9484

About BAK1

BAK, officially called Bcl2 antagonist killer, is a protein that in humans, encoded by the BAK gene. The BAK protein is a pro-apoptotic member of the Bcl-2 gene family which is involved in initiating apoptosis. BAK gene spans 7.6 kb and contains 6 exons. By Southern blot analysis of genomic DNA from human/rodent somatic cell hybrids, BAK gene is localized to chromosome 6. This protein localizes to mitochondria, and functions to induce apoptosis. It interacts with and accelerates the opening of the mitochondrial voltage-dependent anion channel, which leads to a loss in membrane potential and the release of cytochrome. This protein also interacts with the tumor suppressor P53 after exposure to cell stress.

Overview

Product Name	Anti-BAK/BAK1 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-BAK/BAK1 Antibody Picoband® catalog # PB9484. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q16611

Technical Details

Immunogen	E.coli-derived human BAK recombinant protein (Position: A22-S211). Human BAK shares 78.3 % amino acid (aa) sequence identity with mouse BAK.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized



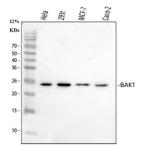




Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Immunohistochemistry (Frozen Section), 0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 2ug/ml Flow Cytometry (Fixed), 1-3ug/1x10 ⁶ cells



Anti-BAK/BAK1 Antibody Picoband® (PB9484) Images



Western blot analysis of BAK using anti-BAK antibody (PB9484).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

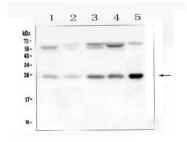
Lane 1: human Hela whole cell lysates,

Lane 2: human 293T whole cell lysates,

Lane 3: human MCF-7 whole cell lysates,

Lane 4: human CACO-2 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-BAK antigen affinity purified polyclonal antibody (Catalog # PB9484) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for BAK at approximately 23 kDa. The expected band size for BAK is at 23 kDa.



Western blot analysis of BAK using anti-BAK antibody (PB9484).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat skeletal muscle tissue lysates,

Lane 2: rat lung tissue lysates,

Lane 3: mouse heart tissue lysates,

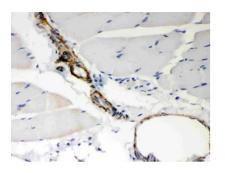
Lane 4: mouse skeletal muscle tissue lysates,

Lane 5: mouse lung tissue lysates.

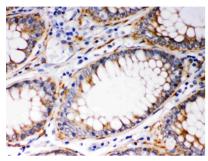
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-BAK antigen affinity purified polyclonal antibody (Catalog # PB9484) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for BAK at approximately 25KD. The expected band size for BAK is at 23KD.

IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in paraffin-embedded section of Rat Skeletal Muscle Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution)

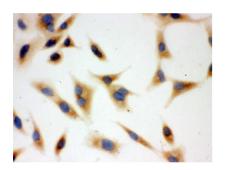




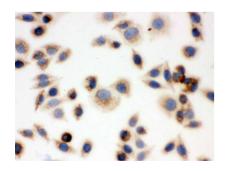
for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in paraffin-embedded section of Human Intestinal Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



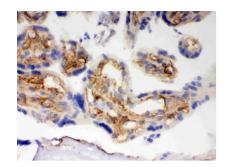
IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in immunocytochemical section of A549 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in immunocytochemical section of SMMC-7721 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog

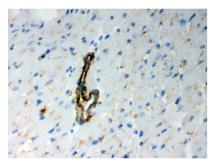




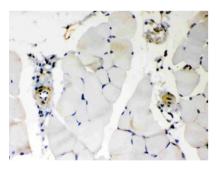
SA1022) with DAB as the chromogen.



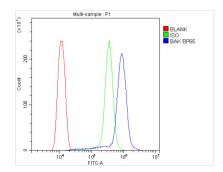
IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in frozen section of mouse cardiac muscle tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in frozen section of rat cardiac muscle tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



IHC analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in paraffin-embedded section of Mouse Skeletal Muscle Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-BAK Antibody (PB9484) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

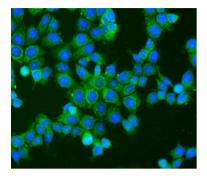


Flow Cytometry analysis of THP-1 cells using anti-BAK antibody (PB9484).

Overlay histogram showing THP-1 cells stained with PB9484 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-BAK Antibody (PB9484,1ug/1x10⁶ cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions.



Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of BAK using anti-BAK antibody (PB9484). BAK was detected in immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-BAK Antibody (PB9484) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

2 Publications Citing This Product

- 1. PubMed ID: 15248898, BAK overexpression mediates p53-independent apoptosis inducing effects on human gastric cancer cells
- 2. PubMed ID: 21573215, Minicircle-oriP-IFN?: A Novel Targeted Gene Therapeutic System for EBV Positive Human Nasopharyngeal Carcinoma

Visit <u>bosterbio.com/anti-bak-picoband-trade-antibody-pb9484-boster.html</u> to see all 2 publications.

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