

Anti-BAK/BAK1 Antibody Picoband™ (monoclonal, 4C2)

Catalog Number: M01163-1

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™ (monoclonal, 4C2)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-BAK/BAK1 Antibody Picoband™ (monoclonal, 4C2) catalog # M01163-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 4C2
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
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	Mouse
Uniprot ID	Q16611

Technical Details

Immunogen	E.coli-derived human BAK/BAK1 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-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b
Form	Lyophilized
Concentration	0
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this



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kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 2ug/ml Flow Cytometry, 1-3ug/1x10 ⁶ cells
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Anti-BAK/BAK1 Antibody Picoband™ (monoclonal, 4C2) (M01163-1) Images

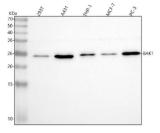


Figure 1. Western blot analysis of BAK/BAK1 using anti-BAK/BAK1 antibody (M01163-1).

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 293T whole cell lysates,

Lane 2: human A431 whole cell lysates,

Lane 3: human THP-1 whole cell lysates,

Lane 4: human MCF-7 whole cell lysates,

Lane 5: human PC-3 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 mouse anti-BAK/BAK1 antigen affinity purified monoclonal antibody (Catalog # M01163-1) 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-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for BAK/BAK1 at approximately 25 kDa. The expected band size for BAK/BAK1 is at 23 kDa.

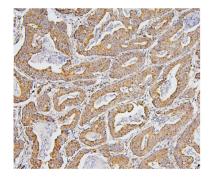


Figure 2. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of human intestinal cancer tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

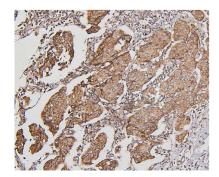


Figure 3. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of human mammary cancer tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.





Figure 4. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of human tonsil tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

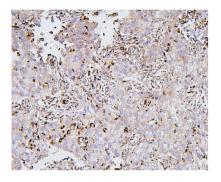


Figure 5. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of human lung cancer tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

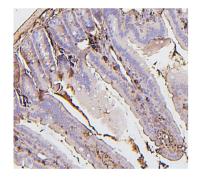


Figure 6. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of mouse intestine tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

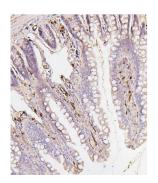
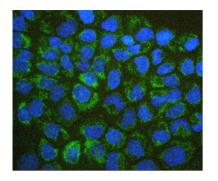


Figure 7. IHC analysis of BAK1 using anti-BAK1 antibody (M01163-1).

BAK1 was detected in paraffin-embedded section of rat intestine tissues. 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 mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

Figure 8. IF analysis of BAK1 using anti-BAK1 antibody (M01163-1).





BAK1 was detected in immunocytochemical section of A431 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 2ug/mL mouse anti-BAK1 Antibody (M01163-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-mouse IgG (BA1126) 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.

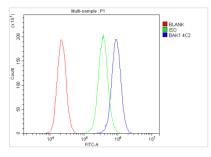


Figure 9. Flow Cytometry analysis of PC-3 cells using anti-BAK1 antibody (M01163-1).

Overlay histogram showing PC-3 cells stained with M01163-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-BAK1 Antibody (M01163-1,1ug/1x10 6 cells) for 30 min at 20 $^\circ$ C. DyLight® 488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10 6 cells) was used as secondary antibody for 30 minutes at 20 $^\circ$ C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10 6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

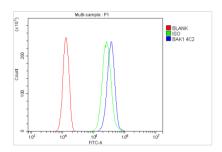


Figure 10. Flow Cytometry analysis of SiHa cells using anti-BAK1 antibody (M01163-1).

Overlay histogram showing SiHa cells stained with M01163-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-BAK1 Antibody (M01163-1,1ug/1x10 6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10 6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10 6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

1 Publications Citing This Product

1. PubMed ID: 15248898, BAK overexpression mediates p53-independent apoptosis inducing effects on human gastric cancer cells

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