

# Anti-Bid Antibody Picoband™ (monoclonal, 10F4)

Catalog Number: M00730-1

#### **About BID**

BID (BH3-Interacting Domain Death Agonist), is a pro-apoptotic member of the Bcl-2 protein family. The BCL2 family of proteins consists of both antagonists and agonists that regulate apoptosis and compete through dimerization. By fluorescence in situ hybridization, Wang et al. (1998) mapped the human BID gene to 22q11. Luo et al. (1998) reported the purification of a cytosolic protein that induces cytochrome c release from mitochondria in response to caspase-8, the apical caspase activated by cell surface death receptors such as FAS and TNF.

#### Overview

Product Name	Anti-Bid Antibody Picoband™ (monoclonal, 10F4)
Reactive Species	Human
Description	Boster Bio Anti-Bid Antibody Picoband™ (monoclonal, 10F4) catalog # M00730-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 10F4
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
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	P55957

### **Technical Details**

Immunogen	E.coli-derived human Bid recombinant protein (Position: M1-D195). Human Bid shares 64% and 61% amino acid (aa) sequences identity with mouse and rat Bid, respectively.
Predicted Reactive Species	Hepatitis Virus
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	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.



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Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this 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, Human  Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human  Immunocytochemistry/Immunofluorescence, 5ug/ml, Human  Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells, Human



## Anti-Bid Antibody Picoband™ (monoclonal, 10F4) (M00730-1) Images

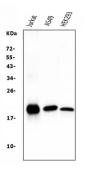


Figure 1. Western blot analysis of Bid using anti-Bid antibody (M00730-1).

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 50ug of sample under reducing conditions.

Lane 1: human Jurkat whole cell lysates;

Lane 2: human A549 whole cell lysates;

Lane 3: human HEK293 whole cell 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 mouse anti-Bid antigen affinity purified monoclonal antibody (Catalog # M00730-1) 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 Bid at approximately 22KD. The expected band size for Bid is at 22KD.

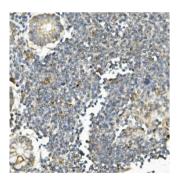


Figure 2. IHC analysis of Bid using anti-Bid antibody (M00730-1).

Bid was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-Bid Antibody (M00730-1) 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.

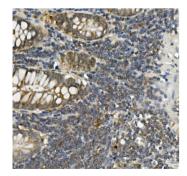


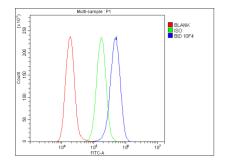
Figure 3. IHC analysis of Bid using anti-Bid antibody (M00730-1).

Bid was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-Bid Antibody (M00730-1) 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. Flow Cytometry analysis of A549 cells using anti-Bid antibody (M00730-1).

Overlay histogram showing A549 cells stained with





M00730-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Bid Antibody (M00730-1,  $1ug/1x10^6$  cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10<sup>6</sup> 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.

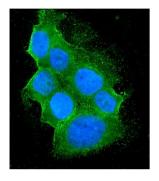


Figure 5. IF analysis of Bid using anti-Bid antibody (M00730-1).

Bid 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 5ug/mL mouse anti-Bid Antibody (M00730-1) 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.

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