

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

Catalog # ABO14959

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P55957
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Bid Antibody Picoband™ (monoclonal, 10F4) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	637
Other Names	BH3-interacting domain death agonist, p22 BID, BID, BH3-interacting domain death agonist p15, p15 BID, BH3-interacting domain death agonist p13, p13 BID, BH3-interacting domain death agonist p11, p11 BID, BID
Calculated MW	21995
Application Details	Western blot, 0.1-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Subcellular Localization	Mitochondrion membrane. Mitochondrion outer membrane. Cytoplasm.
Tissue Specificity	Isoform 2 and isoform 3 are expressed in spleen, bone marrow, cerebral and cerebellar cortex. Isoform 2 is expressed in spleen, pancreas and placenta (at protein level). Isoform 3 is expressed in lung, pancreas and spleen (at protein level). Isoform 4 is expressed in lung and pancreas (at protein level).
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 10F4
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.

Purification	Immunogen affinity purified.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	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.

Protein Information

Name	BID
Function	Induces caspases and apoptosis (PubMed: 14583606). Counters the protective effect of BCL2 (By similarity).
Cellular Location	Cytoplasm. Mitochondrion membrane. Mitochondrion outer membrane. Note=When uncleaved, it is predominantly cytoplasmic. [BH3-interacting domain death agonist p13]: Mitochondrion membrane {ECO:0000250 UniProtKB:P70444}. Note=Associated with the mitochondrial membrane. {ECO:0000250 UniProtKB:P70444} [Isoform 3]: Cytoplasm
Tissue Location	[Isoform 2]: Expressed in spleen, pancreas and placenta (at protein level). [Isoform 4]: Expressed in lung and pancreas (at protein level).

Background

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.

Images

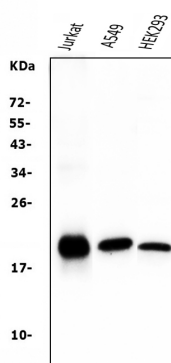


Figure 1. Western blot analysis of Bid using anti-Bid antibody (M00730-1).

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 µg/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.

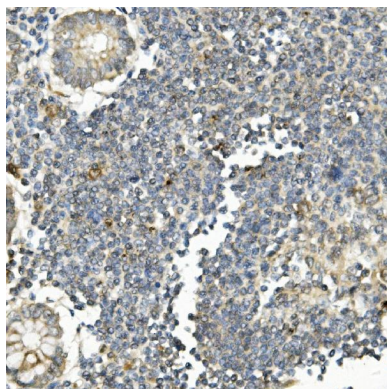


Figure 2. IHC analysis of Bid using anti-Bid antibody (M00730-1).

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 1 $\mu\text{g/ml}$ 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

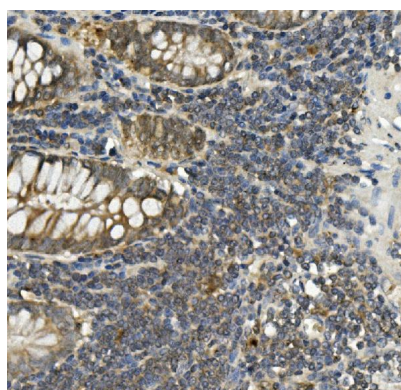


Figure 3. IHC analysis of Bid using anti-Bid antibody (M00730-1).

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 1 $\mu\text{g/ml}$ 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

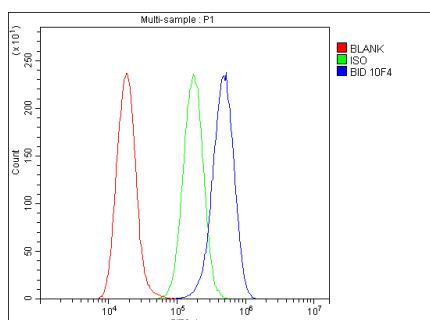


Figure 4. Flow Cytometry analysis of A549 cells using anti-Bid antibody (M00730-1).

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, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

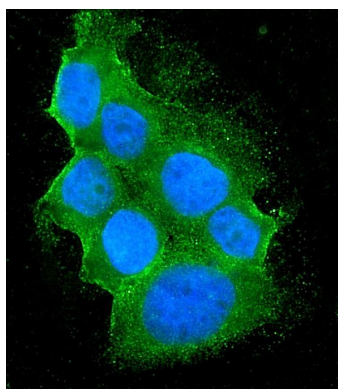


Figure 5. IF analysis of Bid using anti-Bid antibody (M00730-1).

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 5 $\mu\text{g/mL}$ 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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