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Monoclonal Antibody to Bax - Azide Free

Alternate names: Apoptosis regulator BAX, BCL2-associated X protein, BCL2L4, Bcl-2-like protein 4, Bcl2-L-4

Catalog No.: AM00283AF-N

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: Bax (Bcl-associated X protein) is a 21 kDa tumor suppressor protein that suppresses tumorigenesis and stimulates apoptosis in vivo. Bax has extensive amino acid homology to Bcl-2. It can homodimerize through its BH3 domain and it forms heterodimers with other Bcl-2 family members through its BH1 and BH2 domains. Overexpression of Bax promotes apoptosis and counters the death repressor activity of Bcl-2 and Bcl-xL. It is believed that the ratio of Bcl-2/Bax complexes to free protein controls the relative susceptibility of cells to death stimuli. Apoptotic stimuli cause the translocation of monomeric Bax from the cytosol to the mitochondria where it forms Bax homodimers. Localization of Bax to the mitochondria results in the activation of caspase-3, membrane blebbing, and nuclear fragmentation. Bax also induces mitochondrial dysfunction by increasing mitochondrial membrane permeability. It accelerates the opening of the mitochondrial porin channel VDAC, thus regulating the release of cytochrome c during apoptosis.

Uniprot ID: Q07813

NCBI: NP_031553

GeneID: 12028

Host / Isotype: Mouse / IgG1

Clone: 5B7

Immunogen: Synthetic peptide (KLH conjugated) corresponding to amino acids 3–16 near the N-terminus of Mouse Bax.
AA Sequence:
GSGEQLGSGGPTSS
Remarks: The immunogen sequence is not present in Human or Rat Bax.
Hybridoma was established by fusion of mouse myeloma
cell NS-1 with Balb/c mouse splenocyte.

Format: **State:** Liquid purified IgG fraction.
Purification: Protein-A Sepharose Chromatography of hybridoma supernatant.
Buffer System: PBS, pH 7.2 containing 50% Glycerol without preservatives.

Applications: **ELISA.**
Western blotting: 1 µg/mL for chemiluminescence detection system.
Immunofluorescence.
Immunoprecipitation: 2 µg/200 µL of cell extract from 5x10e6 cells.
Detailed procedure is provided in **Protocols.**

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with Mouse Bax (21 kDa) on Western blotting.

Species Reactivity: **Tested:** Mouse.

Add. Information: This product was originally produced by MBL International.

Storage: Store the antibody (in aliquots) at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Hsu Y.T., et al. J. Biol. Chem. 272, 13829-13834 (1997).
Clone 5B7 is used in this reference.

Protocols:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10%glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 POD-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 3 minutes.
Develop the film as usual. The condition for exposure and development may vary.
Positive Controls: NIH/3T3, BaF/3,
WR19L

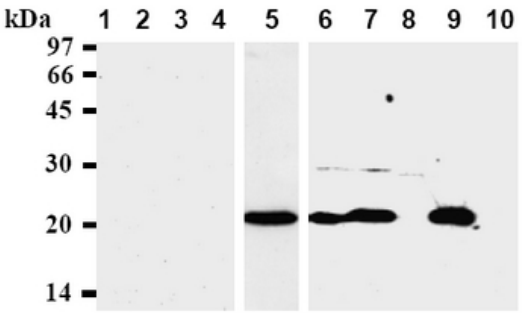
Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 200 µL of the supernatant. Mix

well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting.)**
Positive Contro: WR19L

Pictures:

Western blot analysis of Bax expression in HL60 cells (Lane 1), Raji cells (Lane 2), MCF7 cells (Lane 3), A431 cells (Lane 4), WR19L cells (Lane 5), C2C12 cells (Lane 6), NIH/3T3 cells (Lane 7), Rat-1 cells (Lane 8), BaF/3 cells (Lane 9) and PC12 cells (Lane 10) using Bax antibody AM00283AF-N



Immunoprecipitation of Bax and Bcl-xL from WR19L cells with AM00283AF-N (Lane 2 and Lane 6), Mouse IgG1 (Lane 3) or Mouse IgG2a (Lane 5). After immunoprecipition with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM00283AF-N (Lane 1, 2 and 3) or BcL-xL monoclonal antibody AM20181AF-N (Lane 4, 5 and 6). WR19L crude lysate was resolved in Lane 1 and 4.