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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.: AM20182AF-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: Q07812

NCBI: NP_004315

GeneID: 581

Host / Isotype: Mouse / IgG1

Clone: 6A7

Immunogen: Synthetic peptide KLH conjugated corresponding to N-terminal sequence of Bax
AA Sequence:
CGPTSSEQIMKTGA

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: **Immunoprecipitation:** 2 µg/200 µL of cell extract from 5x10e6 cells.
Addition of nonionic detergent is necessary (Triton X-100 or NP-40 is recommended).
Positive Controls: Jurkat, WR19L cells.
Note: It is reported that this monoclonal antibody can be used in Immunocytochemistry (Ref.3).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with Bax (25 kDa) on Immunoprecipitation.

Species Reactivity: **Tested:** Human and 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. Hou, Q., and Hsu, Y. T. Am. J. Physiol. Heart Circ. Physiol. 289, H477-H487 (2005)
2. Yethon, J. A., et al., J. Biol. Chem. 278, 48935-48941 (2003)
3. Thomenius, M. J., et al., J. Biol. Chem. 278, 6243-6250 (2003)
4. Hsu, Y. T., et al., J. Biol. Chem. 272, 10777-10783 (1998)
5. Hsu, Y. T., et al., J. Biol. Chem. 272, 13829-13834 (1997) Clone 6A7 is used in these references.

Protocols:

<u>Immunoprecipitation</u>

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.
6) Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
7) 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.
8) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at RT, or overnight at 4°C.
9) Incubate the membrane with 1 µg/mL of anti-Bax antibody AM20182AF-N as primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with the 1:5,000 HRP-conjugated anti-mouse IgG heavy chain antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
12) Wash the membrane with PBS-T (5 minutes x 6 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
14) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
15) Expose to an X-ray film in a dark room for 5 minutes.
16) Develop the film as usual. The condition for exposure and development may vary.
Positive Contro: Jurkat, WR19L

Pictures:

Immunoprecipitation of Baxfrom WR19L with AM20182AF-N (Lane 1) or Mouse IgG1 (Lane 2). After immunoprecipitation with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM20182AF-N