For Research Use Only.
Not for use in diagnostic procedures.

MBL

MONOCLONAL ANTIBODY

Anti-BAX (Human) mAb

Code No.	Clone	Subclass	Quantity	Form
M010-3	4F11	Mouse IgG2b κ	100 µg	Lyophilized

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.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 4F11) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with recombinant human BAX.

FORMULATION: This antibody is lyophilized form. Prepare a stock solution by dissolving the lyophilized antibody in 100 µL of distilled water. After reconstitution, the IgG concentration should be 1 mg/mL in PBS (pH 7.2) containing 1% sucrose. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of shipment when stored at 4°C. After reconstitution, avoid repeated freezing and thawing. For storage, prepare appropriate aliquots and freeze them at -20°C.

REACTIVITY: This antibody reacts with human BAX (24 kDa) on Western blotting.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

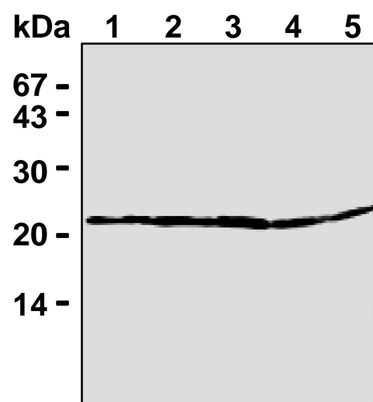
Species	Human	Mouse	Rat
Cells	A431, HEp-2, HeLa, Raji, MRC-5	WR19L	Not tested
Reactivity on WB	+	-	

APPLICATIONS:
Western blotting; 0.1-1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5-10 µg/200 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; 10 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; 10 µg/mL
Flow cytometry; 5-10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

REFERENCES:
1) Cartron, P-F., *et al.*, *J. Biol. Chem.* **280**, 10587-10598 (2005)
2) Starczynski, J., *et al.*, *J. Clin. Oncol.* **23**, 1514-1521 (2005)
3) Suárez, L., *et al.*, *Clin. Cancer Res.* **10**, 7599-7606 (2004)
4) Utsunomiya, K., *et al.*, *J. Histochem. Cytochem.* **52**, 805-812 (2004)
5) Cartron, P-F., *et al.*, *J. Biol. Chem.* **278**, 11633-11641 (2003)
6) Sturlan, S., *et al.*, *Blood* **101**, 4990-4997 (2003)
7) Than, N. G., *et al.*, *Eur. J. Biochem.* **270**, 1176-1188 (2003)
8) Lataillade, J-J., *et al.*, *Blood* **99**, 1117-1129 (2002)
9) Nomura, M., *et al.*, *Cancer Res.* **59**, 5542-5548 (1999)
10) De Saint Jean, M., *et al.*, *Invest. Ophthalmol. Vis. Sci.* **40**, 2199-2212 (1999)
11) Penault-Llorca, F., *et al.*, *Pathol. Res. Pract.* **194**, 457-464 (1998)

Clone 4F11 is used in these references.



Western blot analysis of human BAX expression in A431 (1), HEp-2 (2), MRC-5 (3), HeLa (4) and Raji (5) using M010-3.

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PROTOCOLS:

SDS-PAGE & Western Blotting

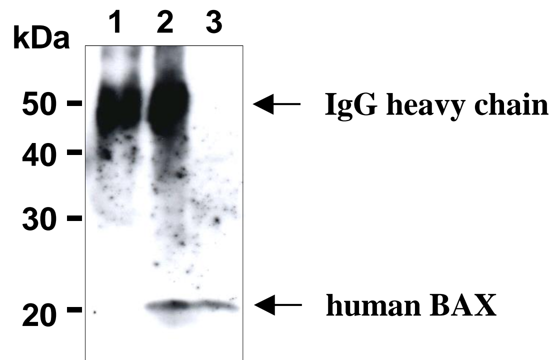
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge 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 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 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% 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 1% skimmed milk (in PBS, pH 7.2) as suggested 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 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
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 for Western blotting; A431, HEp-2, MRC-5, HeLa and Raji)

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 beads 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 control for Immunoprecipitation; Raji)



Immunoprecipitation of human Bax from Jurkat with mouse IgG2b (1) or M010-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M010-3.*

Flow cytometric analysis for cells

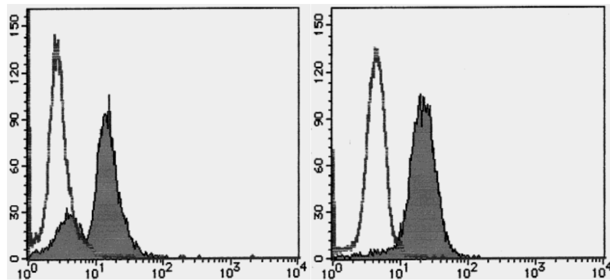
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
3) Wash the cells 3 times with washing buffer.
4) Add 200 µL of 70% ethanol to the cell pellet after tapping. Mix well, then permeablize the cells for 30 minutes at -20°C.
5) Wash the cells 3 times with washing buffer.
6) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 40 µL of the primary antibody diluted with the washing buffer at the concentration as suggested in the **APPLICATIONS**. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add FITC-conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature.

Remove supernatant by careful aspiration.

11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; Jurkat and Raji)

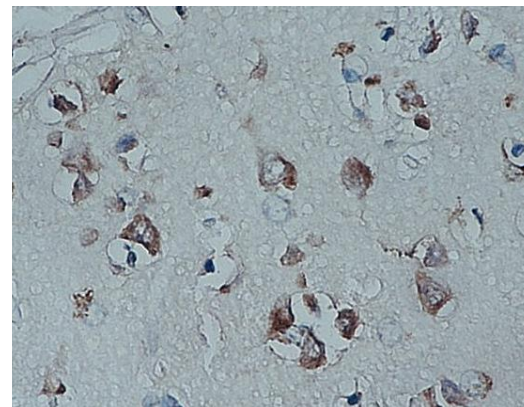


Flow cytometric analysis of human BAX expression on Jurkat (right) and Raji (left). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of M010-3 to the cells.*

Immunohistochemical staining for paraffin embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 2 times for 3-5 minutes each.
3) Wash the slides with 95% Ethanol 2 times for 3-5 minutes each.
4) Wash the slides with deionized water for 30 seconds.
5) Remove the slides from the deionized water and cover each section with 3% H_2O_2 for 5 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Alzheimer's brain)

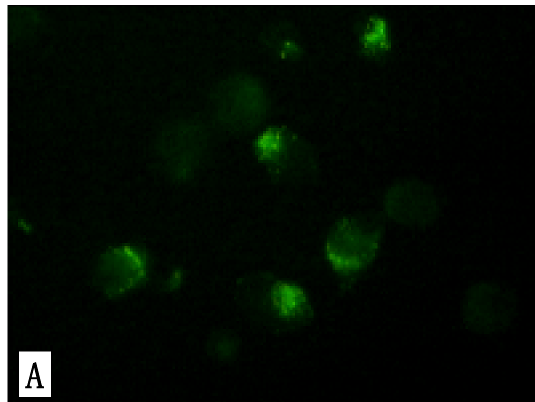


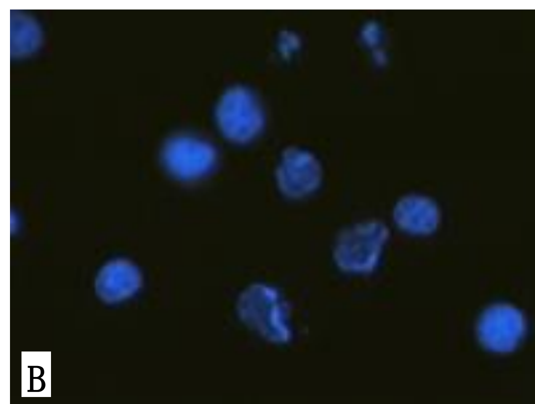
Immunohistochemical detection of human BAX on paraffin embedded section of Alzheimer's brain with M010-3.

Immunocytochemistry

1) Incubate the cells with RPMI1640 containing 10% fetal calf serum (FCS), 5 µg/mL of Actinomycin D for 3 hours at 37°C.
2) Wash the cells with washing buffer [PBS containing 2% FCS and 0.1% NaN_3].
3) Add 200 µL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at 4°C.
4) Wash the cells 3 times with washing buffer.
5) Add 200 µL of Digitonin (100 µg/mL) to the cell pellet after tapping. Mix well, incubate the cells for 10 minutes at room temperature.
6) Wash the cells 3 times with washing buffer.
7) Add 100 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
8) Add 100 µL of the primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**. Mix well and incubate for 30 minutes at room temperature.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Add FITC-conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
12) Counter stain with DAPI (0.2 µg/mL in PBS) for 5 minutes at room temperature.
13) Wash the cells with PBS (5 minutes x 3 times).
14) Resuspend the cells with mounting medium.
15) Drop the cell suspension onto glass slide then put a cover slip on it.

(Positive control for Immunocytochemistry; apoptosis induced HL-60)





Immunocytochemical detection of human BAX on 4% PFA fixed apoptosis induced HL-60 with M010-3 (A) and DAPI (B).

RELATED PRODUCTS:

D038-3	Anti-Bcl-2 mAb (83-8B)
D038-5	Anti-Bcl-2 mAb-PE (83-8B)
M072-3	Anti-BID (Human) mAb (5C9)
591	Anti-Bad pAb (polyclonal)
M030-3	Anti-Bag-1 mAb (4A2)
CM001-1	Anti-Cytochrome c mAb (1E4)