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

MBL
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MONOCLONAL ANTIBODY

Anti-Bcl-2 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D038-3	83-8B	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: The Bcl-2 related genes can inhibit (Bcl-X_L and Mcl-1) or induce (Bax, Bcl-Xs, Bag and Bad) apoptosis in several systems. Bad was identified as a Bcl-2 interacting protein using a yeast two-hybrid screening and λ expression cloning. It has homology to Bcl-2 within the Bcl-2 homolog domains 1 and 2 (BH1 and BH2). In mammalian cells, Bad selectively heterodimerizes with Bcl-X_L as well as Bcl-2, but not with other Bcl-2 family members (Bax, Bcl-Xs, Mcl-1 and A1). When Bad heterodimerized with Bcl-X_L, it displaced Bax from Bcl-X_L and promoted cell death.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell PAI with Balb/c mouse splenocyte immunized with recombinant rat Bcl-2β.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human, mouse and rat Bcl-2 on Western blotting and Flow cytometry.

APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji	WR19L	PC12
Reactivity on WB	+	+	+

INTENDED USE:
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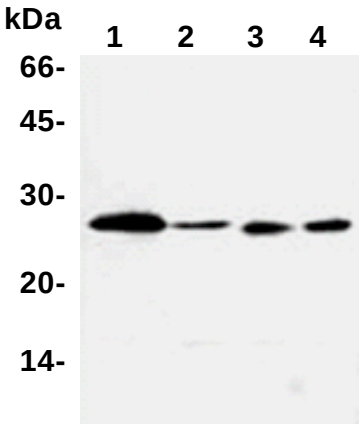
REFERENCES:
1) Viollet, L., *et al., J. Immunol*. **177**, 6685-6694 (2006)
2) Rincheval, V., *et al., Biochem. Biophys. Res. Commun.* **298**, 282-288 (2002) [WB]
3) Tanaka, T., *et al., Eur. Respir. J*. **20**, 359-368 (2002) [WB]
4) Tamatani, M., *et al., J. Biol. Chem.* **274**, 8531-8538 (1999) [WB]
5) Nunez, G., *et al., J. Immunol*. **144**, 3602-3610 (1990)
6) Tsujimoto, Y., *et al., Oncogene* **4**, 1331-1336 (1989)
7) Vaux, D. L., *et al., Nature* **335**, 440-442 (1988)
8) Tsujimoto, Y., *et al., Science* **228**, 1440-1443 (1985)

Clone 83-8B is used in reference number 1) - 4).



Western blotting analysis of Bcl-2 expression in Jurkat (1), Raji (2), WR19L (3) and PC12 using D038-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:
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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
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).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, WR19L and PC12)

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