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Product datasheet for AM26425AF-N

Bcl2 Mouse Monoclonal Antibody [Clone ID: 83-8B]

Product data:

Product Type:	Primary Antibodies
Clone Name:	83-8B
Applications:	FC, WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. Flow cytometry: 10 µg/ml. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant rat Bcl-2β
Specificity:	This antibody reacts with Bcl-2.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	B-cell CLL/lymphoma 2
Database Link:	Entrez Gene 24224 Rat P49950

View online »

Background: The Bcl-2 related genes can inhibit (Bcl-XL 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 ? 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-XL 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-XL, it displaced Bax from Bcl-XL and promoted cell death.

Synonyms: BCL2, Bcl-2 alpha

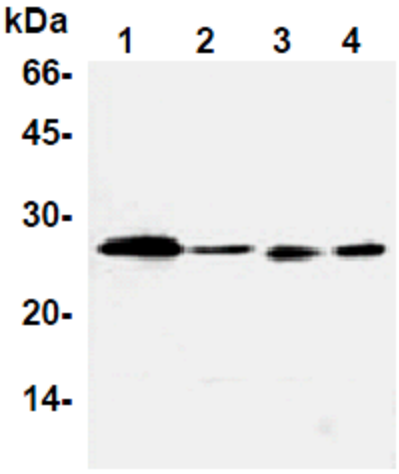
Note: This product was originally produced by MBL International.

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 4oC 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 4oC 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 4oC.
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 HRP-conjugated anti-mouse IgG 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.
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, PC12)

Product images:



Western blot analysis of Bcl-2 expression in Jurkat (1), Raji (2), WR19L (3) and PC12 using AM26425AF-N.