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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-XIAP (MIHA/ILP-a) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M044-3	2F1	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Caspase, related to ICE and to CED-3, plays central roles as effectors of apoptosis. Ablation of caspase activity is attained by p35 from baculovirus and CrmA from cowpox, which appear to be suicide inactivators, strongly inhibiting caspase activity. Overexpression of there caspase inhibitors in insect, nematoda and mammalian cells results in resistance to apoptotic stimuli, demonstrating that components of the apoptotic pathway are highly conserved throughout evolution, and leading to the speculation that mammalian functional equivalents of these protease inhibitors may exist. The inhibitor of apoptosis proteins (IAPs) are a family of anti-apoptotic proteins that are conserved across species. Four IAPs have been identified in mammal; NAIP, cIAP1/HIAP2/hMIHB, cIAP2/HIAP1/hMIHC, XIAP/hILP. A prototype of the human IAPs is the XIAP, with a 1.5 kb coding region corresponding to a 55 kDa protein. XIAP can directly inhibit two members of the cell death protease family, caspase-3 and -7.

SOURCE: This antibody was purified from hybridoma (clone 2F1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the full length recombinant XIAP protein (1-497 aa).

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 XIAP on Western blotting. 2F1 recognizes C-terminal region of XIAP (352-449 aa) and detects 55 kDa of XIAP on Western Blot using total cell lysate from human or mouse cell line, for example, Jurkat, Raji, NIH/3T3, etc.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, U937, Raji, HeLa, HL-60	NIH/3T3, Ba/F3, WR19L	PC12
Reactivity on WB	+	+	weak

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
Immunocytochemistry; Not tested
Flow cytometry; Not tested
*It is reported that this antibody has been used in this application in the reference number 3), 6) and 10)-12).

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

INTENDED USE:

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REFERENCES:

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14) Farahani, R., *et al. Genomics* **42**, 514-518 (1997)
15) Roy, N., *et al., EMBO J.* **16**, 6914-6925 (1997)
16) Deveraux, QL., *et al. Nature* **388**, 300-304 (1997)
17) Liston, P., *et al. Nature* **379**, 349-353 (1996)

Clone 2F1 is used in reference 1) - 12).

PROTOCOL:

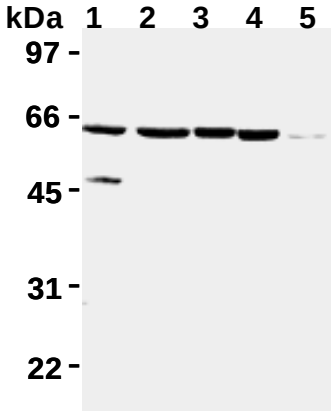
SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volumes 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% MeOH). See the manufacture'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 to be used will depend on the conditions.)
8) Wash the membrane with PBS (10 minutes x 3 times).
9) Incubate the membrane with 1:5,000 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 (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 2 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for western blotting; Jurkat, Raji, HeLa, U937, HL-60, WR19L, NIH/3T3, Ba/F3and PC12)



Western blot analysis of XIAP expression in Jurkat (1), Raji (2), HeLa (3), WR19L(4) and PC12 (5) using M044-3.

For more information, please visit our web site.
http://ruo.mbl.co.jp/