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

MBL

MONOCLONAL ANTIBODY

Anti-Apaf-1 (Human) mAb

Code No.	Clone	Subclass	Quantity	Form
M074-3	5C1	Mouse IgG1	100 µg	solution in 50% Glycerol /PBS

SOURCE: This antibody was purified from cell culture supernatant using protein-A Sepharose. This hybridoma was established by fusion of mouse myeloma P3U1 with Balb/c splenocyte immunized with His-tagged human Apaf-1 fusion protein corresponding to N-terminal amino acids (1-421 a. a.).

STORAGE: This antibody is stable for 3 years from the date of manufacture when stored at -20°C.

PRODUCT: 100 µg of IgG in 100 µL volume of PBS containing 50% glycerol. No preservative is contained.

SPECIFICITY: This antibody detects ~130 kDa of human Apaf-1 protein from whole cell lysate of human cell lines, such as Jurkat, HeLa and U937 on Western blotting.

APPLICATION:

Western blotting ; 5 µg/ml
*Detailed procedure is provided in the following **RESEARCH APPLICATION.**

SPECIES CROSS REACTIVITY :

Species	Human	Mouse	Rat	Others
reactivity	+	+ (weak)	+ (weak)	NT
Cell line	Jurkat, Raji, HeLa, U937, HL60, HPB-ALL	L5178Y, NIH3T3, WR19L	Rat1	

NT; not tested

Entrez Gene :

317 (Human), 11783 (Mouse), 78963 (Rat)

REFERENCES :

1) Pan, G., *et al., J. Biol. Chem.* **237**, 5841-5845 (1998)
2) Yoshida, H., *et al., Cell* **94**, 739-750 (1998)
3) Cecconi, F., *et al., Cell* **94**, 727-737 (1998)
4) Adrain, C., *et al., J. Biol. Chem.* **274**, 20855-20860 (1999)
5) Perkins, C. L., *et al., Cancer Res.* **60**, 1645-1653 (2000)

RESEARCH APPLICATION:

SDS PAGE & Western Blotting

1) Rinse 1×10^7 of cells 3 times with PBS and suspend it with 1ml of 1 × Laemmli SDS-PAGE sample buffer, then lyse the cells by brief sonication (up to 20 sec.).
2) Boil the sample for 3-5 min. and centrifuge at 12,000 × g for a minute. Other methods may be employable.
3) Resolve 20 µl of the sample per lane by 7.5 % SDS-polyacrylamide gel electrophoresis (See the manufacture's manual for electrophoresis condition).
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
5) To reduce nonspecific binding, soak the membrane in Block Ace™ (Snow bland) for 1 hr. at 37°C, or overnight at 4°C.
6) Dilute the primary antibody to 5 µg/ml with Dilution Buffer (10% Block Ace™/PBS). Incubate the membrane with primary antibody dilution for 1 hr. at room temperature (20-25°C). (The concentration of antibody to be used will be dependent on condition.)
7) Wash the membrane with Wash Buffer (0.2% Tween20 in PBS) for 5-10 min. × 3 times.
8) Incubate the membrane with secondary antibody (1:10,000 diluted anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) in Dilution Buffer) for 1 hr. at room temperature.
9) Wash the membrane with Wash buffer (10 min. × 3 times)
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.

M074-3
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1/1

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