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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Human BID

Code No.	Clone	Subclass	Quantity	Concentration
M072-3	5C9	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: Apoptosis is a major form of cell death characterized by several morphological features that include chromatin condensation and fragmentation, cell membrane blebbing, and formation of apoptotic bodies. BID is an apoptosis promoting protein, which possesses only BH3 domain that is a common structure of Bcl-2 family members. Once full length BID (p22) is cleaved with caspases, cleaved BID (p15) induces a conformational change and oligomerization of a protein named BAK on mitochondrial membrane. It is thought that oligomerized BAK contribute to forming pores that release cytochrome *c* into cytosol.

SOURCE: This antibody was purified from hybridoma (clone 5C9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human BID (61-118 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 human BID on Western blotting.

APPLICATIONS:
Western blotting; 1-5 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

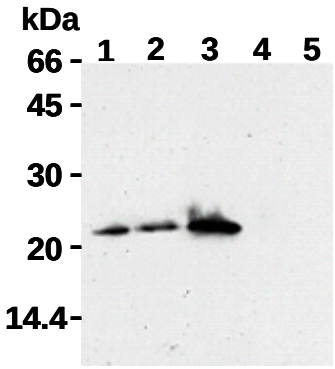
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, Raji, HeLa, ZR-75-1, U937, HL-60, HPB-ALL	WR19L, L5178Y, NIH/3T3	Rat-1	BHK
Reactivity on WB	+	-	-	-

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

REFERENCES:
1) Thiery, J., *et al.*, *J. Immunol.* **174**, 871-878 (2005)
2) Uddin, S., *et al.*, *Clin. Cancer Res.* **11**, 3102-3108 (2005)
3) Baumann, R., *et al.*, *FASEB J.* **17**, 2221-2230 (2003)
4) Eskes, R., *et al.*, *Mol. Cell Biol.* **20**, 929-935 (2000)
5) Perez, D., *et al.*, *Mol. Cell Biol.* **6**, 53-63 (2000)
6) Zha, J., *et al.*, *Science* **290**, 1761-1765 (2000)
7) Korsmeyer, S.J., *et al.*, *Cell Death Differ.* **7**,1166-1173 (2000)
8) Yin, X.M., *et al.*, *Nature* **400**, 886-891 (1999)
9) Luo, X., *et al.*, *Cell* **94**, 481-490 (1998)
10) Li, H., *et al.*, *Cell* **94**, 491-501 (1998)
11) Wang, K., *et al.*, *Genes. Dev.* **10**, 2859-2869 (1996)

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



Western blot analysis of human BID expression in ZR-75-1 (1), U937 (2), HL-60 (3), WR19L (4) and PC12 (5) using M072-3.

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 2 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4^{o}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 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (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 (5 minutes x 6 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 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; ZR-75-1, U937, HL-60)

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.