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

MONOCLONAL ANTIBODY

Anti-Caspase-3 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M087-3	1F9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Caspase-3 (also known as CPP32, Yama, apopain) is a key member of the caspase family of cysteine proteases. Caspase-3 exists in cells as an inactive 32 kDa proenzyme. During apoptosis procaspase-3 is processed at aspartate residues by self-proteolysis and/or cleavage by upstream caspases, such as caspase-6, -8, or -9. The processed form of caspase-3 consists of large (17 kDa) and small (12 kDa) subunits which associate to form the active tetrameric enzyme tetramer (a pair of heterodimers). The active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cells (e.g., PARP, SREBPs, and DFF). Activation of procaspase-3 stands at a point of convergence for the two major types of apoptosis signaling pathways-those linked to cell surface death receptors and those linked to mitochondrial release of cytochrome *c*.

SOURCE: This antibody was purified from hybridoma (clone 1F9) 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 caspase-3.

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 caspase-3 (32 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; 10 µg/mL
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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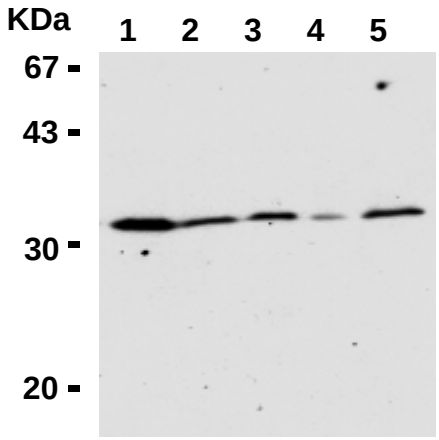
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Raji, HL60, A431, HPB-ALL, KG-1	WR19L, NIH/3T3	PC12, Rat1
Reactivity on WB	+	-	-

REFERENCES:

1) Huang, C. R., *et al.*, *Anticancer Res.* **30**, 2065-2071 (2010) [WB]
2) Yang, L. Q., *et al.*, *World J. Gastroenterol.* **10**, 22-25 (2004)



Western blot analysis of Caspase-3 expression in Raji (1), HL60 (2), A431 (3), HPB-ALL (4) and KG-1 (5) using M087-3.

PROTOCOLS:

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.

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e-mail support@mbl.co.jp, TEL 052-238-1904

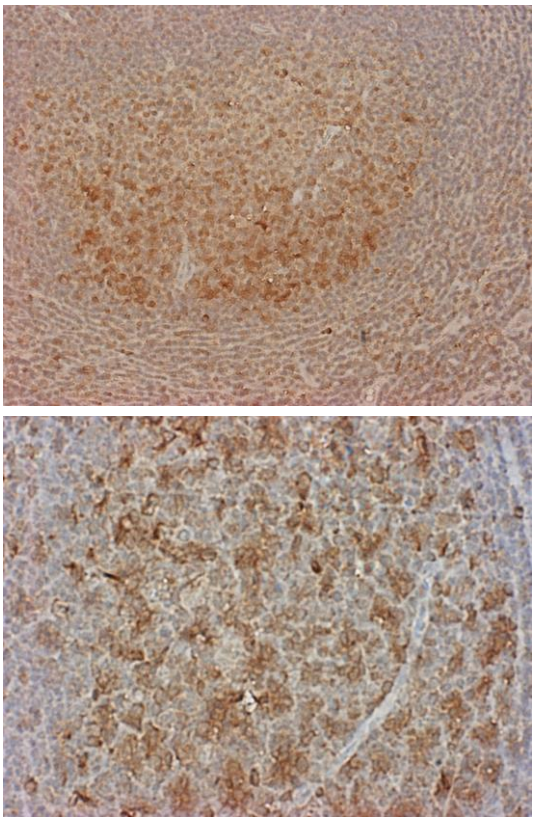
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°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 will depend on conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 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 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; Raji, HL60, A431, HPB-ALL and KG-1)

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunohistochemistry; Human tonsil)



Immunohistochemical detection of caspase-3 on human tonsil paraffin embedded section with M087-3.

RELATED PRODUCTS:

Antibodies

M073-3	Anti-Caspase-2 (Human) mAb (4F8)
M097-3	Anti-Caspase-3 (Human) mAb (1F3)
K0197-3	Anti-Caspase-3 (Human) mAb (AMI-3-1-11)
M087-3	Anti-Caspase-3 (Human) mAb (1F9)
M088-3	Anti-Caspase-3 (Human) mAb (7D12)
M029-3	Anti-Caspase-4 (Human) mAb (4B9)
M060-3	Anti-Caspase-5 mAb (4F7)
M070-3	Anti-Caspase-6 mAb (3E8)
M053-3	Anti-Caspase-7 mAb (4G2)
M032-3	Anti-Caspase-8 (Human) mAb (5F7)
M058-3	Anti-Caspase-8 (Human) mAb (5D3)
M054-3	Anti-Caspase-9 mAb (5B4)
M059-3	Anti-Caspase-10 (Human) mAb (4C1)
K0193-3	Anti-Caspase-14 (Human) mAb (8-1-71)

Kits

4700	MEBCYTO® Apoptosis Kit (Annexin V-FITC Kit)
8445	MEBSTAIN Apoptosis TUNEL Kit Direct
8442	MEBSTAIN Apoptosis TUNEL Kit III
4800	APOPCYTO Caspase-3 Colorimetric Assay Kit
4805	APOPCYTO Caspase-8 Colorimetric Assay Kit
4810	APOPCYTO Caspase-9 Colorimetric Assay Kit
4815	APOPCYTO Caspase-3 Fluorometric Assay Kit
4820	APOPCYTO Caspase-8 Fluorometric Assay Kit
4825	APOPCYTO Caspase-9 Fluorometric Assay Kit