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

MONOCLONAL ANTIBODY

Anti-Caspase-3

Code No.	Clone	Subclass	Quantity	Concentration
M097-3	1F3	Rat IgG2a	100 µg	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 pro-caspase-3 is processed at aspartate residues by self-proteolysis and/or cleavage by upstream caspases, such as caspases-6, 8, or 9. The processed form of caspases-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 caspases-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cells (e.g., PARP, SREBPs, and DFF). Activation of pro-caspase-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 1F3) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat splenocyte immunized with the recombinant full-length 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 pro-caspase-3 (32 kDa) and active caspase-3 (17 kDa) on Western blotting.

APPLICATIONS:

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

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

INTENDED USE:

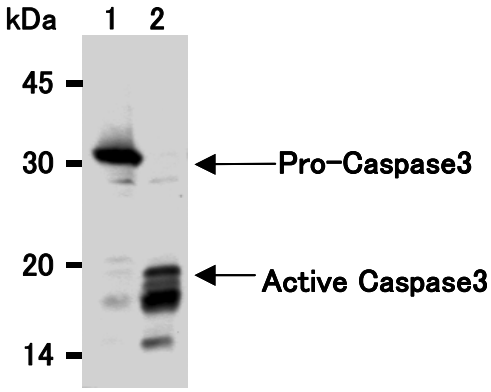
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Apoptotic Jurkat	Not Tested	Not Tested
Reactivity on WB	+		

REFERENCE:

1) Yang, L.Q., *et al., World J. Gastroenterol.* **10**, 22-25 (2004)



Western blot analysis of Caspase3 expression in Jurkat cells treated with anti-Fas antibody (SY-001) 0hr (1) or 4hr (2) using M097-3.

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 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 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 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rat IgG (code no. IM-0825) 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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat and apoptotic Jurkat)

RELATED PRODUCTS:

M073-3	Anti-Caspase-2 (4F8)
K0197-3	Anti-Caspase-3 (AMI-3-1-11)
M087-3	Anti-Caspase-3 (1F9)
M088-3	Anti-Caspase-3 (7D12)
M029-3	Anti-Caspase-4 (4B9)
M060-3	Anti-Caspase-5 (4F7)
M070-3	Anti-Caspase-6 (3E8)
M053-3	Anti-Caspase-7 (4G2)
M032-3	Anti-Caspase-8 (5F7)
M058-3	Anti-Caspase-8 (5D3)
M054-3	Anti-Caspase-9 (5B4)
M059-3	Anti-Caspase-10 (4C1)
K0206-3	Anti-Caspase-12 (14F7)
K0207-3	Anti-Caspase-12 (14F4)
K0193-3	Anti-Caspase-14 (8-1-71)
4690	APOPCYTO Annexin V-Azami-Green Apoptosis Detection Kit
4700	MEBCYTO Apoptosis Kit
8445	MEBSTAIN Apoptosis TUNEL Kit Direct
8441	MEBSTAIN Apoptosis TUNEL Kit Ⅱ
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
4817	Intracellular Caspase-3 Activity Detection Kit
4822	Intracellular Caspase-8 Activity Detection Kit
4827	Intracellular Caspase-9Activity Detection Kit
4830	APOPCYTO Intracellular Caspases Activity Detection Kit