

CHICKEN MONOCLONAL ANTIBODY

Anti-Cytochrome *c*

Code No.	Clone	Subclass	Quantity
CM001-1	1E4	Chicken IgY	1 mL

BACKGROUND: Cytochrome *c* released from mitochondria bind to Apaf-1 (Apoptotic protease activating factor-1). In the presence of dATP, Apaf-1/cytochrome *c* complex is induced its conformational change that exposes the caspase recruitment domain (CARD) of Apaf-1, allowing caspase-9 to bind. Activated caspase-9 in turn cleaves and activates caspase-3. Activated caspase-3 cleaves poly(ADP-ribose) polymerase, DNA-dependent protein kinase, nuclear lamins and several other cellular proteins, leading to apoptosis.

SOURCE: This antibody was concentrated from hybridoma (clone 1E4) supernatant. This hybridoma was established by fusion of chicken B cell line MuH1 cell with chicken splenocyte immunized with the synthetic peptide, CKEERADLIAYLKKATNE which corresponding to the C-terminus of human cytochrome *c*.

FORMULATION: 1 mL aliquot of concentrate antibody from the supernatant with preservative (0.09% sodium azide).

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with cytochrome *c* (15 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1:10 for chemiluminescence detection system

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

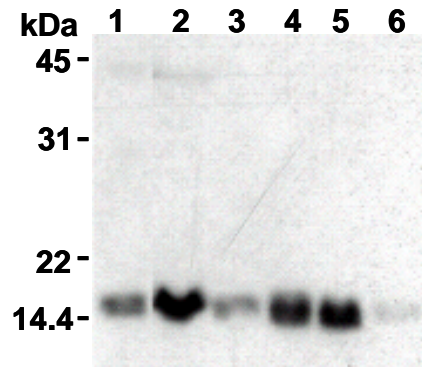
Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, HeLa, Raji	NIH/3T3	Rat-1	CHO
Reactivity on WB	+	+	+	+

INTENDED USE:

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



Western blot analysis of cytochrome *c* expression in Jurkat (1), HeLa (2), Raji (3), NIH/3T3 (4), CHO (5) and Rat-1 (6) using CM001-1.

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 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/cm² 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 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 1:5,000 HRP-conjugated anti-chicken IgY (MBL; code no. PM010-7) 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; Jurkat, HeLa, Raji, NIH/3T3, CHO, Rat-1)

RELATED PRODUCTS:

- | | | | |
|---------|--|---------|--|
| M073-3 | Anti-Caspase-2 (4F8) | D057-3 | Anti-Mouse Fas ligand (FLIM58) |
| M097-3 | Anti-Caspase-3 (1F3) | D057-4 | FITC labeled Anti-Mouse Fas ligand (FLIM58) |
| K0197-3 | Anti-Caspase-3 (AMI-3-1-11) | D057-6 | Biotin labeled Anti-Mouse Fas ligand (FLIM58) |
| M087-3 | Anti-Caspase-3 (1F9) | D069-3 | Anti-Mouse Fas ligand (FLIM4) |
| M088-3 | Anti-Caspase-3 (7D12) | D086-3 | Anti-ASC (23-4) |
| M029-3 | Anti-Caspase-4 (4B9) | D132-3 | Anti-PD-1 (J110) |
| M060-3 | Anti-Caspase-5 (4F7) | D132-4 | FITC labeled Anti-PD-1 (J110) |
| M070-3 | Anti-Caspase-6 (3E8) | D133-3 | Anti-PD-1 (J105) |
| M053-3 | Anti-Caspase-7 (4G2) | D161-3 | Anti-MFG-E8 (2422) |
| M032-3 | Anti-Caspase-8 (5F7) | D199-3 | Anti-MFG-E8 (18A2-G10) |
| M058-3 | Anti-Caspase-8 (5D3) | D184-3 | Anti-Granulysin (RB1) |
| M054-3 | Anti-Caspase-9 (5B4) | D185-3 | Anti-Granulysin (RC8) |
| M059-3 | Anti-Caspase-10 (4C1) | D185-6 | Biotin labeled Anti-Granulysin (RC8) |
| K0206-3 | Anti-Caspase-12 (14F7) | D186-3 | Anti-Granulysin (RF10) |
| K0207-3 | Anti-Caspase-12 (14F4) | D200-3 | Anti-Human BAFF/BLyS (1D6) |
| K0193-3 | Anti-Caspase-14 (8-1-71) | D200-4 | FITC labeled Anti-Human BAFF/BLyS (1D6) |
| M010-3 | Anti-Bax (4F11) | D201-3 | Anti-Human BAFF-R/BR3 (8A7) |
| M028-3 | Anti-Mouse TRAF1 (3D4) | D201-4 | FITC labeled Anti-Human BAFF-R/BR3 (8A7) |
| M030-3 | Anti-Bag-1 (4A2) | K0033-3 | Anti-DR3 (B65) |
| M031-3 | Anti-TRADD (3E11) | K0033-4 | FITC labeled Anti-DR3 (B65) |
| M033-3 | Anti-FADD (1F7) | K0039-3 | Anti-TNF-R1 (H398) |
| M035-3 | Anti-FADD (4G3) | K0039-4 | FITC labeled Anti-TNF-R1 (H398) |
| M037-3 | Anti-DFF45/ICAD (6B8) | K0040-3 | Anti-TNF-R2 (80M2) |
| M044-3 | Anti-XIAP (2F1) | K0040-4 | FITC labeled Anti-TNF-R2 (80M2) |
| M056-3 | Anti-RAIDD (4B12) | K0127-3 | Anti-Daxx (DAXX-01) |
| M072-3 | Anti-BID (5C9) | K0145-3 | Anti-CD30 (Ber-H2) |
| M074-3 | Anti-Apaf-1 (5C1) | K0145-4 | FITC labeled Anti-CD30 (Ber-H2) |
| M083-3 | Anti-AcinusL (3H8) | K0151-3 | Anti-Bax (5B7) |
| M112-3 | Anti-Mouse TRAF2 (6F8) | K0152-3 | Anti-Bax (6A7) |
| D026-3 | Anti-Mouse Fas (CD95) (RMF2) | K0153-3 | Anti-Bcl-xL (2H12) |
| D027-3 | Anti-Mouse Fas (CD95) (RMF6) | K0154-3 | Anti-Bcl-2 (10C4) |
| D038-3 | Anti-Bcl-2 (83-8B) | K0157-3 | Anti-IKK γ (I- κ B Kinase γ) (DA10-12) |
| D038-5 | PE labeled Anti-Bcl-2 (83-8B) | K0159-3 | Anti-IKK γ (I- κ B Kinase γ) (EA2-6) |
| D041-3 | Anti-Human Fas ligand (4H9) | K0194-3 | Anti-HtrA2/Omi (18-1-83) |
| D041-4 | FITC labeled Anti-Human Fas ligand (4H9) | PM004 | Anti-Smac/DIABLO (Polyclonal) |
| D041-5 | PE labeled Anti-Human Fas ligand (4H9) | PD005 | Anti-Vimentin Fragment (V1) (Polyclonal) |
| D041-6 | Biotin labeled Anti-Human Fas ligand (4H9) | PD006 | Anti-SET β (p41/p42) (Polyclonal) |
| D042-3 | Anti-Human Fas ligand (4A5) | PD007 | Anti-SET β (p42) (Polyclonal) |
| | | PD008 | Anti-SET β (p41) (Polyclonal) |
| | | 591 | Anti-Bad (Polyclonal) |
| | | 592 | Anti-Mouse TRAF2 (Polyclonal) |
| | | 597 | Anti-Mouse TRAF6 (Polyclonal) |
| | | 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-9 Activity Detection Kit |
| | | 4830 | APOPCYTO Intracellular Caspases Activity Detection Kit |