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

MONOCLONAL ANTIBODY

Anti-Human RAIDD

Code No.	Clone	Subclass	Quantity	Concentration
M056-3	4B12	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. These morphological changes occur via signaling pathway that leads to the recruitment and activation of caspases, a family of cysteine-containing, aspartate-specific proteases. The coupling of caspases to signaling pathway is probably mediated by adaptor molecules that contain protein-protein interaction motifs. For example, in the TNF receptor I mediated apoptosis pathway, the apoptotic signal is thought to be transduced through interaction of the oligomerized receptor death domain (DD) with a set of cytoplasmic adaptor/signaling molecules including FADD, TRADD and RIP. RAIDD (also known as CRADD) is another candidate for such adaptor molecules. It has a bipartite architecture comprising a carboxy-terminal DD and an amino-terminal caspase recruitment domain (CARD) highly homologous with the sequence of the prodomain of human caspase-2 and *C. elegans* CED-3. RAIDD interacts with RIP on respective DD, and with caspase-2 or CED-3 on respective CARD. Thus it may play a role in the TNF-receptor I mediated apoptosis by recruiting caspase-2 to the pathway. However, it has been remained unclear about the significance of caspase-2 activation through the RIP/RAIDD pathway for death induction at least in some situation, because targeted disruption of RIP in mice, as well as mutational ablation of RIP function in cultured cells did not interfere with death induction by TNF nor by Fas, while NF-κB activation through RIP were abolished in these cells.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant full-length human RAIDD (200 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 RAIDD (~22 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL 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, Raji, HeLa	WR19L, Ba/F3	PC12, Rat-1	BHK
Reactivity on WB	+	-	-	-

INTENDED USE:

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REFERENCES:

1) Milleron, R. S., *et al., J. Biol. Chem.* **281**, 16991-17000 (2006)
2) Hlaing, T., *et al., J. Biol. Chem.* **276**, 9230-9238 (2001)
3) Chou, J.J., *et al., Cell* **94** 171-180 (1998)
4) Cryns, V., *et al., Genes Dev.* **12**, 1551-1570 (1998)
5) Kovalenko, W.D., *et al., Curr.Opin. Immunol.* **10**, 279-288 (1998)
6) Cohen, G.M., *et al., Biochem. J.* **326**, 1-16 (1997)
7) Duan, H., *et al., Nature* **385**, 86-89 (1997)
8) Ahmad, M., *et al., Cancer Res.* **57** 615-619 (1997)
9) Arends, M. J., *et al., Int. Rev. Exp. Pathol.* **32**, 223-254 (1991)

Clone 4B12 is used in the reference 1) and 2).

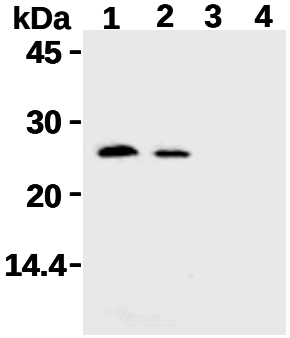
PROTOCOL:

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/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] (10 minutes x 3 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 (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)



Western blot analysis of RAIDD expression in Jurkat (1), HeLa (2), WR19L (3) and PC12 (4) using M056-3.

RELATED PRODUCTS:

M073-3 Anti-Caspase-2 (4F8)
M097-3 Anti-Caspase-3 (1F3)
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)
M010-3 Anti-Bax (4F11)
M028-3 Anti-Mouse TRAF1 (3D4)
M030-3 Anti-Bag-1 (4A2)
M031-3 Anti-TRADD (3E11)
M033-3 Anti-FADD (1F7)
M035-3 Anti-FADD (4G3)
M037-3 Anti-DFF45/ICAD (6B8)
M044-3 Anti-XIAP (2F1)
M072-3 Anti-BID (5C9)
M074-3 Anti-Apaf-1 (5C1)
M083-3 Anti-AcinusL (3H8)
M112-3 Anti-Mouse TRAF2 (6F8)
D026-3 Anti-Mouse Fas (CD95) (RMF2)
D027-3 Anti-Mouse Fas (CD95) (RMF6)
D038-3 Anti-Bcl-2 (83-8B)
D038-5 PE labeled Anti-Bcl-2 (83-8B)
D041-3 Anti-Human Fas ligand (4H9)
D041-4 FITC labeled Anti-Human Fas ligand (4H9)
D041-5 PE labeled Anti-Human Fas ligand (4H9)
D041-6 Biotin labeled Anti-Human Fas ligand (4H9)
D042-3 Anti-Human Fas ligand (4A5)
D057-3 Anti-Mouse Fas ligand (FLIM58)
D057-4 FITC labeled Anti-Mouse Fas ligand (FLIM58)
D057-6 Biotin labeled Anti-Mouse Fas ligand (FLIM58)
D069-3 Anti-Mouse Fas ligand (FLIM4)
D086-3 Anti-ASC (23-4)
D132-3 Anti-PD-1 (J110)
D132-4 FITC labeled Anti-PD-1 (J110)
D133-3 Anti-PD-1 (J105)
D161-3 Anti-MFG-E8 (2422)
D199-3 Anti-MFG-E8 (18A2-G10)
D184-3 Anti-Granulysin (RB1)
D185-3 Anti-Granulysin (RC8)
D185-6 Biotin labeled Anti-Granulysin (RC8)
D186-3 Anti-Granulysin (RF10)
D200-3 Anti-Human BAFF/BLyS (1D6)
D200-4 FITC labeled Anti-Human BAFF/BLyS (1D6)
D201-3 Anti-Human BAFF-R/BR3 (8A7)
D201-4 FITC labeled Anti-Human BAFF-R/BR3 (8A7)
K0033-3 Anti-DR3 (B65)
K0033-4 FITC labeled Anti-DR3 (B65)
K0039-3 Anti-TNF-R1 (H398)
K0039-4 FITC labeled Anti-TNF-R1 (H398)
K0040-3 Anti-TNF-R2 (80M2)
K0040-4 FITC labeled Anti-TNF-R2 (80M2)

K0127-3	Anti-Daxx (DAXX-01)
K0145-3	Anti-CD30 (Ber-H2)
K0145-4	FITC labeled Anti-CD30 (Ber-H2)
K0151-3	Anti-Bax (5B7)
K0152-3	Anti-Bax (6A7)
K0153-3	Anti-Bcl-xL (2H12)
K0154-3	Anti-Bcl-2 (10C4)
K0157-3	Anti-IKKγ (I-κB Kinase γ) (DA10-12)
K0159-3	Anti-IKKγ (I-κB Kinase γ) (EA2-6)
K0194-3	Anti-HtrA2/Omi (18-1-83)
CM001-1	Anti-Cytochrome c (1E4)
PM004	Anti-Smac/DIABLO (Polyclonal)
PD005	Anti-Vimentin Fragment (V1) (Polyclonal)
PD006	Anti-SETβ (p41/p42) (Polyclonal)
PD007	Anti-SETβ (p42) (Polyclonal)
PD008	Anti-SETβ (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-9Activity Detection Kit
4830	APOPCYTO Intracellular Caspases Activity Detection Kit