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MBL
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MONOCLONAL ANTIBODY

Anti-ASC (TMS1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D086-3	23-4	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: ASC (apoptosis-associated speck-like protein containing a CARD (caspase recruitment domain)) is a 22 kDa soluble protein, located in the cytosol of HL-60 cells. In apoptotic HL-60 cells, it is able to be visualized as a speck, forming an insoluble aggregate. The C-terminal domain of this protein contains a CARD, suggesting that ASC may have proapoptotic activity in HL-60 cells. Recent data have indicated that ASC plays a role of adaptor protein linking various PAAD (Pyrin, AIM, ASC, and death domain-like) family proteins to pathways involved in NF-κB and procaspase-1 activation.

SOURCE: This antibody was purified from hybridoma (clone 23-4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3-X63-AG8.653 with Balb/c mouse splenocyte immunized with Triton X-100 insoluble fraction of retinoic acid-treated HL-60 cells.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

REACTIVITY: This antibody reacts with human ASC (22 kDa) on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/300 µL of cell extract from 5 x 10^6 HL-60 cells
Immunohistochemistry; Not tested*
*It is reported that this monoclonal antibody can be used in immunohistochemistry in the reference number 8).
Immunocytochemistry; Not tested**
**It is reported that this monoclonal antibody can be used in immunocytochemistry in the reference number 1), 4) and 11).
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species		Human	Mouse	Rat
Reactivity on WB	+	U937, HL-60		
	-	Jurkat, HeLa, NB4, HPB-ALL	NIH/3T3, L5178Y, WR19L	PC12

INTENDED USE:
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REFERENCES:

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4) Terasawa, K., *et al., Clin. Cancer Res.* **10**, 2000-2006 (2004) [IC]
5) Wang, Y., *et al., Int. Immunol.* **16**, 777-786 (2004)
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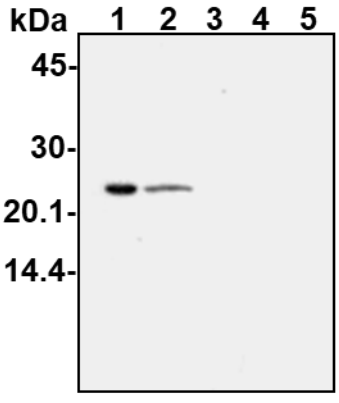
8) Masumoto, J., *et al., J. Histochem. Cytochem.* **49**, 1269-1275 (2001) [IHC]
9) Masumoto, J., *et al., Exp. Cell Res.* **262**, 128-133 (2001)
10) Masumoto, J., *et al., Biochem. Biophys. Res. Commun.* **280**, 652-655 (2001)
11) Masumoto, J., *et al., J. Biol. Chem.* **274**, 33835-33838 (1999) [IC]

Clone 23-4 is used in these references.



Western blotting analysis of ASC expression in U937 (1), HL-60 (2), Jurkat (3), WR19L(4) and PC12 (5) using D086-3.

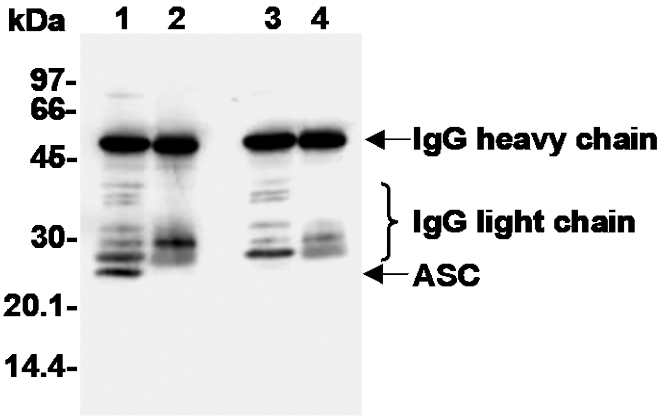
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM HEPES, pH 7.4, 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 6 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 (12.5% acrylamide) 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% Methanol). 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 condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with the 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).
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; U937, HL-60)



Immunoprecipitation of ASC from HL-60 with D086-3 (1, 3) or normal mouse IgG (2, 4). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D086-3 (1, 2) or normal mouse IgG (3, 4).*

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM HEPES, pH 7.4, 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.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 1-4 hour(s) at 4°C.
4) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
5) Resuspend the beads with 1 mL of 1% Triton X-100/PBS.
6) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 3-5 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HL-60)

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