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

MBL
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MONOCLONAL ANTIBODY

Anti-Ash (Grb2) mAb

Code No.	Clone	Subclass	Quantity	Concentration
MS-20-3	3F2	Mouse IgG1 κ	100 μL	1 mg/mL

BACKGROUND: Grb2 (Growth factor receptor bound protein 2) also known as Ash, is a 25-28 kDa molecule composed of central Src homology (SH2) domain and SH3 domains. Grb2 associates with tyrosine phosphorylated proteins such as EGFR, PDGFR, IRS-1, Shc and Gab1 via its SH2 domain. The SH3 domain of Grb2 binds to Sos, which stimulates the GTP binding activity of Ras, cause activation of MAP kinase and other signaling pathways. Grb2 is an adaptor protein, which does not have enzymatic activity itself. Grb2 express ubiquitously and has two isoforms.

SOURCE: This antibody was purified from hybridoma (clone 3F2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1with Balb/c splenocyte immunized with the recombinant Ash (Grb2).

FORMULATION: 100 μg IgG in 100 μL 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 N-terminal SH3 domain of recombinant Ash (Grb2) on Western blotting.

APPLICATIONS:

Western blotting; 2-10 μg/mL
Immunoprecipitation; Not tested*
*This antibody can be used in this application in the reference number 6).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested
Immunofluorescence; Not tested*
*This antibody can be used in this application in the reference number 1).

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Jurkat, HeLa, U937	WR19L	PC12
Reactivity on WB	+	+	+

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11) Gale, N. W., *et al., Nature* **363**, 88-92 (1993)
12) Matuoka, K., *et al., PNAS*. **89**, 9015-9019 (1992)
13) Lowenstein, E. J., *et al., Cell* **70**, 431-442 (1992)
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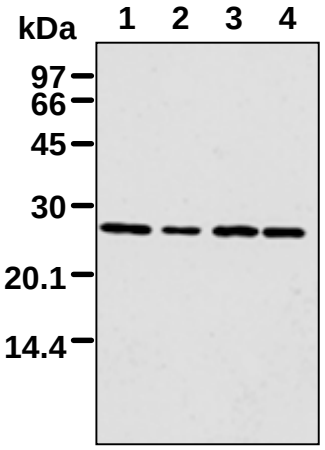
Clone 3F2 is used in reference number 1)-7).



Western blotting analysis of Ash/Grb2 expression in Jurkat (1), HeLa (2), U937 (3) and PC12 (4) using MS-20-3.

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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer'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 the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with 1:10,000 of 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; Jurkat, HeLa, U931, WR19L, PC12 and 3Y1)

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