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

MBL

MONOCLONAL ANTIBODY

Anti-HS1 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M001-3	3A3	Mouse IgG1	100 µL	1mg/mL

BACKGROUND: The HS1 protein is one of the major substrates of non-receptor-type protein-tyrosine kinases and is phosphorylated immediately after crosslinking of the surface IgM on B cells. It is reported that HS1 protein plays a crucial role in the B-cell antigen receptor-mediated signal transduction pathway that leads to apoptosis.

SOURCE: This antibody was purified from hybridoma (clone 3A3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human HS1.

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, mouse and rat HS1 (75 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 10 µg/500 µL of cell extract from 5×10^6 cells
Immunohistochemistry; Not determined
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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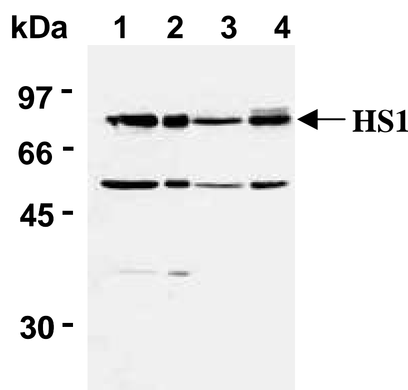
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Raji, Jurkat	WR19L	PC12
Reactivity on WB	+	+	+

REFERENCES:

1) Bunnell, S. C., *et al., J. Biol. Chem.* **275**, 2219-2230 (2000)
2) Hutchcroft, J. E., *et al., J. Immunol.* **161**, 4506-4512 (1998)
3) Suzuki, Y., *et al., J. Immunol.* **158**, 2736-2744 (1997)
4) Fukuda, T., *et al., PNAS* **92**, 7302-7306 (1995)
5) Yamanashi, Y., *et al., PNAS* **90**, 3631-3635 (1993)

Clone 3A3 is used in reference number 2).



Western blot analysis of HS1 expression in Raji (1), Jurkat (2), WR19L (3) and PC12 (4) using M001-3.

PROTOCOLS:

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 protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 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,

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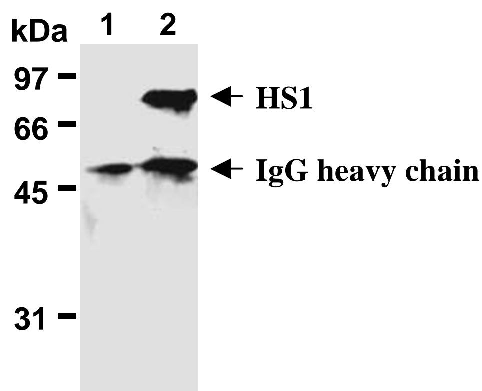
20% MeOH). See the manufacturer's manual for precise transfer procedure.

6) To reduce nonspecific binding, soak the membrane in 5% 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 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 times).
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 (5 minutes x 6 times).
11) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
13) Expose the membrane onto an X-ray film in a dark room for 3 minutes.
14) Develop the film under usual settings. The conditions for exposure and development may vary

(Positive controls for Western blotting; Raji, Jurkat, WR19L and PC12)



Immunoprecipitation of HS1 from Jurkat with mouse IgG1 (1) or M001-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M001-3.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
3) Add 50 µL of 50% protein A agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another fresh two tubes.
5) Add the mouse IgG1 isotype control antibody (MBL; code no. M075-3) or Anti-HS1 mAb (M001-3) at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gentle agitation for 60-120 minutes at 4°C.
6) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
7) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
8) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)