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Product datasheet for AM26574AF-N

HCLS1 Mouse Monoclonal Antibody [Clone ID: 3A3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3A3
Applications:	IP, WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. Immunoprecipitation: 10 µg/500µl of cell extract from 5x10^6 cells.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant human HS1
Specificity:	This antibody reacts with HS1
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Predicted Protein Size:	75 kDa
Gene Name:	hematopoietic cell-specific Lyn substrate 1
Database Link:	Entrez Gene 3059 Human P14317

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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.

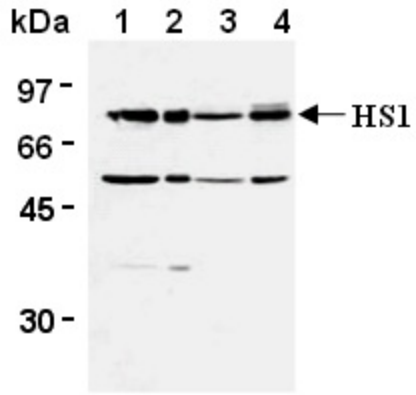
Synonyms: LckBP1

Note: This product was originally produced by MBL International.

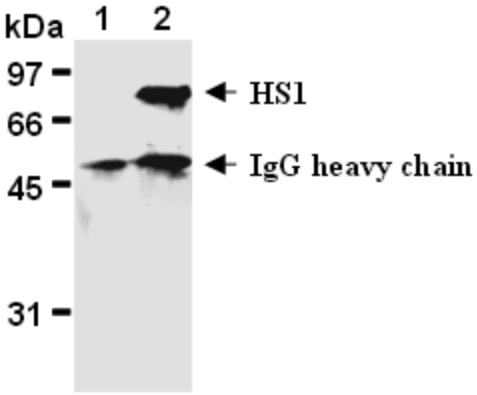
Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing protease inhibitors at appropriate concentrations. Incubate it at 4 o C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 o 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 µ L 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% 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 o 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 the 1:10,000 HRP-conjugated anti-mouse IgG 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 appr opriate 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, PC12)
Immunoprecipitation
1) Wash cells (approximately 1 x 10e7 cells) 3 times with PBS and resuspend them in 1000 µ L 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 o 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 o C and transfer the supernatant to another fresh tube.
3) Add 50 µ L of 50% protein A agarose beads in the supernatant. Incubate it at 4 o C with rotating for 60 minutes.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4 o C. Supernatant is equally divided into another fresh two tube.
5) Add the mouse IgG1 isotype control antibody or anti-HS1 (3A3) antibody at the amount of as suggested in the APPLICATIONS to the supernatant. Vortex briefly and incubate with gently agitation for 60-120 minutes at 4 o C.
6) Add 20 µ L of 50% protein A agarose beads into the tube. Mix well and incubate with gently agitation for 30-60 minutes at 4 o 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 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting .)
(Positive control for Immunoprecipitation; Jurkat)

Product images:



Western blot analysis of HS1 expression in Raji (1), Jurkat (2), WR19L (3) and PC12 (4) using AM26574AF-N.



Immunoprecipitation of HS1 from Jurkat with mouse IgG1 (1) or AM26574AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26574AF-N.