Datasheet for ABIN5541285

anti-HCLS1 antibody

Go to Product page

Overview

Quantity:	0.1 mg
Target:	HCLS1
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This HCLS1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP)

Product Details

Immunogen:	Recombinant human HS1
Clone:	3A3
Isotype:	IgG1
Specificity:	This antibody reacts with HS1
Purification:	Protein A agarose

Target Details

Target:	HCLS1
Alternative Name:	hcls1 (HCLS1 Products)
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

Target Details

	reported that HS1 protein plays a crucial role in the B-cell antigen receptor-mediated signal transduction pathway that leads to apoptosis.
UniProt:	P14317
Pathways:	Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Regulation of Actin Filament Polymerization, Myometrial Relaxation and Contraction, Maintenance of Protein Location

Application Details

Application Notes:	Western blot: 1 µg/mL for chemiluminescence detection system. Immunoprecipitation: 10 µ g/500 µL of cell extract from 5x10 6 cells.
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)

Application Details

	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 gentle 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)
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS containing 50 % glycerol, pH 7.2. No preservative is contained.
Preservative:	Azide free
Storage:	-20 °C
Storage Comment:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.