

Datasheet for ABIN5541066

anti-BAG1 antibody (AA 1-220)



Overview

Quantity:	0.1 mg
Target:	BAG1
Binding Specificity:	AA 1-220
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This BAG1 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	Recombinant full-length mouse Bag-1 (1-220 aa)
Clone:	4A2
Isotype:	lgG1
Specificity:	This antibody react with Bag-1.

Target Details

Target:	BAG1
Alternative Name:	bag1 (BAG1 Products)
Background:	Bcl-2 and its family members control cell death. Bcl-2, Bcl-xL and Mcl-1 inhibit apoptosis, while
	Bad, Bax, Bak, Bik and Bcl-xS promote apoptosis in several conditions. Bag-1 cDNA was

identified as a Bcl-2 binding protein, which lacks sequence homology with Bcl-2, and its related proteins. Bag-1 enhances the anti-apoptotic activity of Bcl-2. Bag-1 specifically interacts with Raf-1 kinase in vitro and in yeast two hybrid assays. It suggests that Bag-1 joins Ras and 14-3-3 proteins as potential activators of the Raf-1 kinase. Moreover Bag-1 binds to the intracellular domain of the hepatocyte growth factor (HGF) receptor, sugges ting that enhances protection from apoptosis. These suggest that Bag-1 represents a new type of anti-cell death gene and that routes of apoptosis induction previously ascribed to Bcl-2 independent pathway may instead reflect a need for the combination of Bcl-2 and Bag-1.

UniProt:

Q60739

Application Details

Application Notes:

Western blot: 1 μ g/mL for chemiluminescence detection system. For details see protocol below.

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 o 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 o 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 3 minutes and centrifuge. Load 10 µ L of the sample per lane in a 1 mm thick SDSpolyacrylamide gel 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 % MeOH). See the manufact ure'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 o C. 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1 % skimmed milk as suggest 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 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 (10 minutes x 3 times). 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

Application Details

	development may vary. (Positive controls for Western blotting HeLa, HL-60, WR19L, PC12)
Restrictions:	For Research Use only
Handling	
Format:	Liquid
Buffer:	Buffer System: Protein A agarose, 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.