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Product datasheet for AM26490AF-N

SSH3BP1 (ABI1) Mouse Monoclonal Antibody [Clone ID: 1B9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1B9
Applications:	IHC, IP, WB
Recommended Dilution:	Western blot: 1 µg / ml for chemiluminescence detection system. Immunoprecipitation: 2 µg / 200 µL of cell extract from 5x10^6 cells. Immunohistochemistry on paraffin sections: 5 µg / ml. Heat treatment is necessary for paraffin embedded sections. Microwave oven; 2 times for 10 minutes each in citrate buffer (pH 6.5). For details see protocol below. This antibody was used in Immunocytochemistry in ref. 1.
Reactivity:	Hamster, Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Human recombinant Abi-1 expressed in E. coli
Specificity:	This antibody reacts with Abi-1.
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 (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Predicted Protein Size:	65 kDa
Gene Name:	abl interactor 1
Database Link:	Entrez Gene 10006 Human Q8IZP0

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Background: Abl interactor (Abi) proteins are c-Abl-binding proteins that bind to both the Src homology 3 (SH3) domain and the C-terminal proline-rich regions of Abl kinase through dual SH3-PXXP interactions. Two highly related genes, abi-1 and abi-2, were cloned. In addition to the interaction with Abl kinase, Abi proteins also interact with other signaling molecules. Recently, it has been reported that Abi-1 dramatically promoted c-Abl-mediated tyrosine (Tyr296) phosphorylation of Mena [mammalian homologue of Drosophila Enabled (Ena)] by interacting with both proteins.

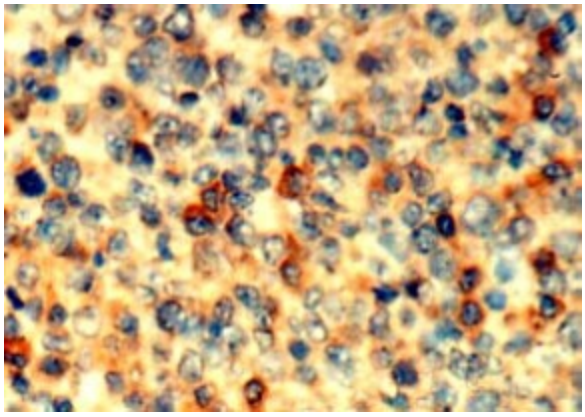
Synonyms: Abl interactor 1, Abelson interactor 1, Abi-1, e3B1, Spectrin binding protein, Nap1-binding protein, Abl-binding protein 4, AblBP4

Note: This product was originally produced by MBL International.

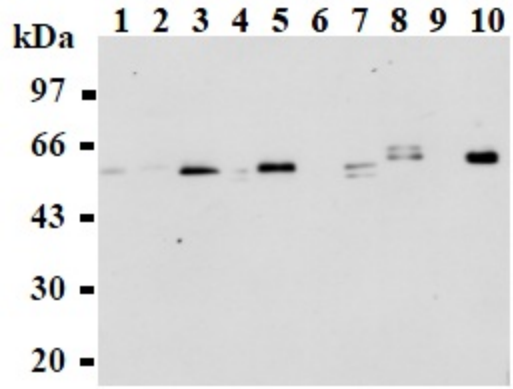
Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 oC 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 the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for specific 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 oC.
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 optimal antibody concentration will depend on the experimental conditions.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 POD-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 (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.
Positive controls for Western blotting; A431, Raji
Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 oC 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 oC and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 oC. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 oC.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
Positive control for Immunoprecipitation; Raji
Immunohistochemical staining for paraffin-embedded sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment by microwave oven: Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H2O2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the APPLICATIONS.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9.
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 9.
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H2O2 in 150 mL PBS.
*DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting. Positive control for Immunohistochemistry; Tonsil

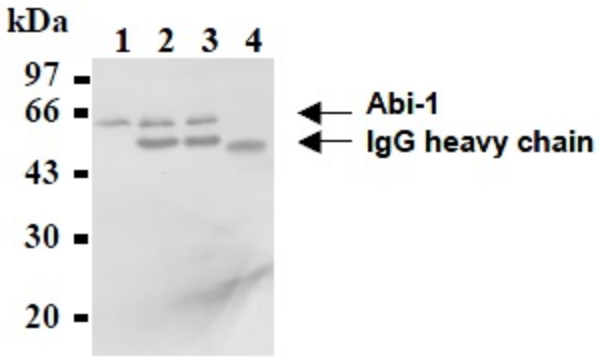
Product images:



Immunohistochemical detection of Abi-1 on paraffin embedded section of a human lung cancer with AM26490AF-N.



Western blot analysis of Abi-1 expression in HeLa cells (1), 293T cells (2), Raji cells (3), Jurkat cells (4), A431 cells (5), U251 cells (6), KG1 cells (7), NIH/3T3 cells (8), WR19L cells (9) and CHO cells (10) using AM26490AF-N.



Immunoprecipitation of Abi-1 from Raji cells with AM26490AF-N (2,3) or mouse IgG (4). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-Abi-1 monoclonal antibody (AM26490AF-N). Raji crude lysate was resolved in lane 1.