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

MONOCLONAL ANTIBODY

Anti-Abi-1 (Abl interactor 1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D147-3	1B9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Abl interactor (Abi) proteins were identified as a c-Abl-binding protein 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 (Tyr^{296}) phosphorylation of Mena [mammalian homologue of *Drosophila* Enabled (Ena)] by interacting with both proteins.

SOURCE: This antibody was purified from hybridoma (clone 1B9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Balb/c mouse splenocyte immunized with human recombinant Abi-1.

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 Abi-1 (65 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system

Immunoprecipitation; 2 µg/ 200 µL of cell extract from $5x10^6$ cells

Immunohistochemistry; 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)

Immunocytochemistry; Not tested*

*It is reported that this antibody can be used in Immunocytochemistry in the reference number 2), 4), 6) and 9).

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*	Hamster	Others**
Cells	A431, Raji	NIH/3T3	Not tested	CHO	
Reactivity on WB	+	+		+	

*The cross reactivity to rat Abi-1 is reported in the reference number 5) and 6).

**The endogenous expression of Abi-1 on MDCK cells has been reported in the reference number 8).

REFERENCES:

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6) Liebau, S., *et al.*, *PLoS One* **6**, e18148 (2011) [WB, IC]
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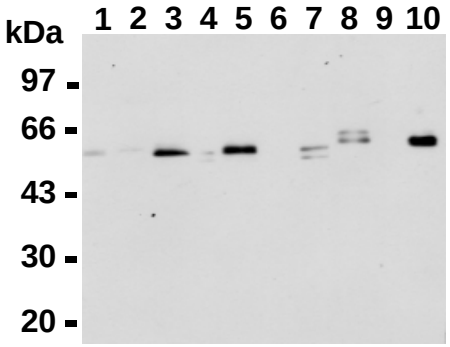

Western blot analysis of Abi-1 expression in HeLa cells (1), 293T cells (2), Raji cells (3), Jurkat cells (4), A431 cells (5), U251 cells (6), KG1 cells (7), NIH/3T3 cells (8), WR19L cells (9) and CHO cells (10) using D147-3

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PROTOCOLS:

SDS-PAGE & Western Blotting

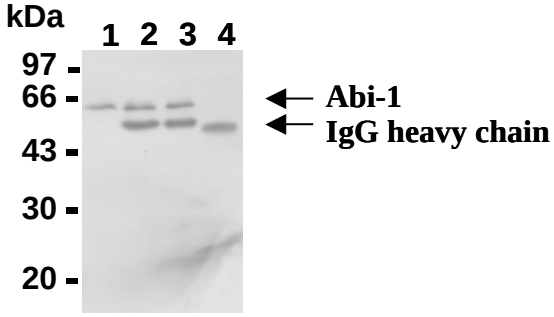
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°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 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/cm^2 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 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°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 the condition.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with 1:10,000 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 (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; A431, Raji, NIH/3T3 and CHO)

Immunoprecipitation

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°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°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 1 hour at 4°C.
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)



Immunoprecipitation of Abi-1 from Raji cells with D147-3 (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 (D147-3). Raji crude lysate was resolved in lane 1*

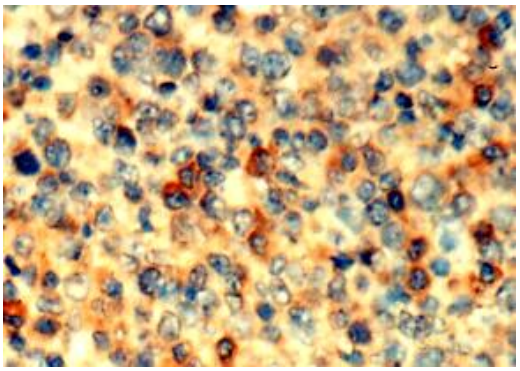
Immunohistochemical staining for paraffin-embedded sections : SAB method

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
   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% H_2O_2 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 (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) 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 suggest 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 (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 9.
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). 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% H_2O_2 in 150 mL PBS. *DAB is a suspect 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)



Immunohistochemical detection of Abi-1 on paraffin embedded section of a human lung cancer with D147-3.

RELATED PRODUCTS:

RN019P Anti-HNRNPK pAb
M075-3 Mouse IgG1 (isotype control) (2E12)