

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 (Tyr²⁹⁶) 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 5x10⁶ 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:

For research use only. Not for clinical diagnosis.

SPECIES CROSS REACTIVITY:

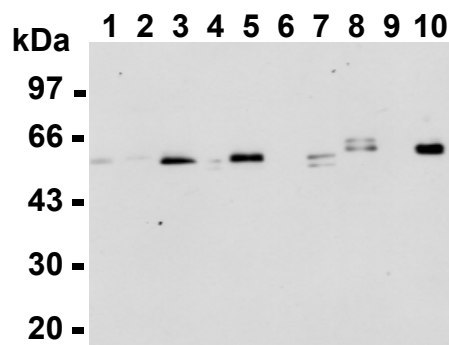
Species	Human	Mouse	Rat*	Hamster	Others**
Cells	A431, Raji	NIH/3T3	Not tested	CHO	
Reactivity on WB	+	+		+	

*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:

- 1) Sekino, S., *et al.*, *Cell Commun. Signal.* **13**, 41 (2015) [IP]
- 2) Steinestel, K., *et al.*, *Mol. Cancer* **13**, 145 (2014) [WB, IC, IHC]
- 3) Xiong, X., *et al.*, *Oncogenesis* **1**, e26 (2012) [WB]
- 4) Steinestel, K., *et al.*, *PLoS One* **7**, e40671 (2012) [WB, IC, IHC]
- 5) Proepper, C., *et al.*, *PLoS One* **6**, e27045 (2011) [WB]
- 6) Liebau, S., *et al.*, *PLoS One* **6**, e18148 (2011) [WB, IC]
- 7) Eto, K., *et al.*, *Blood* **110**, 3637-3447 (2007) [WB]
- 8) Yamazaki, D., *et al.*, *J. Cell Sci.* **120**, 86-100 (2007) [WB]
- 9) Kheir, W.A., *et al.*, *J. Cell Sci.* **118**, 5369-5379 (2005) [WB, IP, IC]
- 10) Oda, A., *et al.*, *Blood* **105**, 3141-3148 (2005) [WB, IP]
- 11) Tani K., *et al.*, *J. Biol. Chem.*, **278**, 21685-21692 (2003)



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

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/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 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)

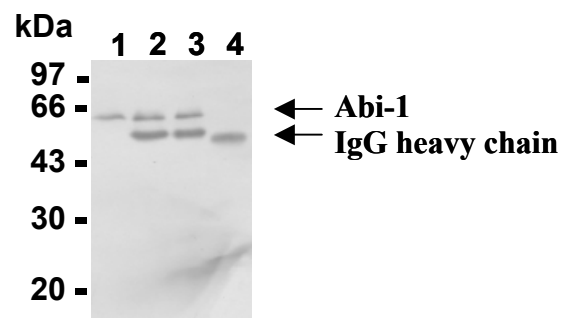
Immunoprecipitation

- 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

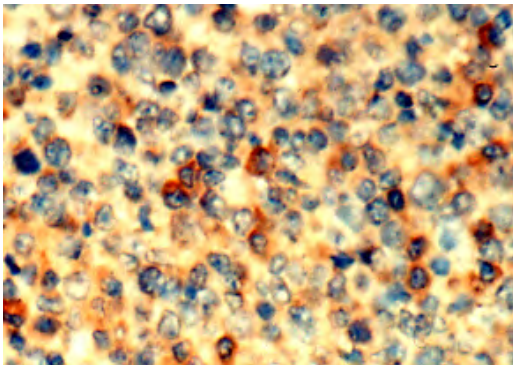
Immunohistochemical staining for paraffin-embedded sections : SAB method

- 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% 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 (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 μ L of 30% H₂O₂ 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)