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Not for use in diagnostic procedures.

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POLYCLONAL ANTIBODY

Anti-ATBF1 (AT-6) pAb

Code No.	Quantity	Form
PD011	100 µL	Affinity Purified

BACKGROUND: ATBF1 (also known as ZFHX3) is a transcription factor that has two-protein isoforms, the 404 kDa ATBF1-A and the 306 kDa ATBF1-B. ATBF1-A contains four homeodomains and 23 zinc-finger motifs. ATBF1-B contains four homeodomains and 18 zinc fingers. ATBF1 is identified as DNA-binding protein, which binds to an AT-rich element of the human α-fetoprotein (AFP) gene, as a result suppressing its transcription activity. ATBF1 is also involved in cell cycle arrest and cooperating with p53 to activate the $p21^{Waf1/Cip1}$ promoter. ATBF1 is expressed in the differentiation fields in association with β-tubulin III and MAP2 that are the neuronal differentiation marker. ATBF1 plays a crucial role in neuronal development and cell cycle arrest.

SOURCE: This antibody was purified from rabbit serum by affinity column chromatography. The rabbit was immunized with recombinant C-terminus region of human ATBF1 corresponding to 3405-3459 aa.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. Contains no preservative.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with C-terminus region of ATBF1 on Western blotting and Immunoprecipitation.

APPLICATIONS:
Western blotting; 1:1,000-1:5000
Immunoprecipitation; 2 µL/300-500 µg of protein
Immunohistochemistry; Not recommended
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedures are provided in the following **PROTOCOLS**.

INTENDED USE:
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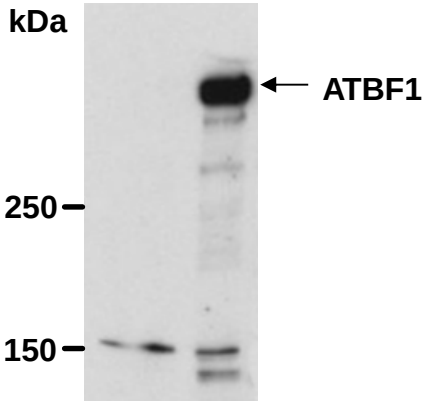
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Lu99A, T24, HeLa, A549	P19 (differentiated)	embryo (E14) brain
Reactivity on WB	+	+	+

REFERENCES:
1) Jung, C. G., *et al.*, *Development* **132**, 5137-5145 (2005)
2) Zhang, Z., *et al.*, *Clin. Cancer Res.* **11**, 193-198 (2005)
3) Ishii, Y., *et al.*, *J. Comp. Neurol.* **465**, 57-71 (2003)
4) Berry, F. B., *et al.*, *J. Biol. Chem.* **276**, 25057-25065 (2001)
5) Miura, Y., *et al.*, *J. Biol. Chem.* **270**, 26840-26848 (1995)



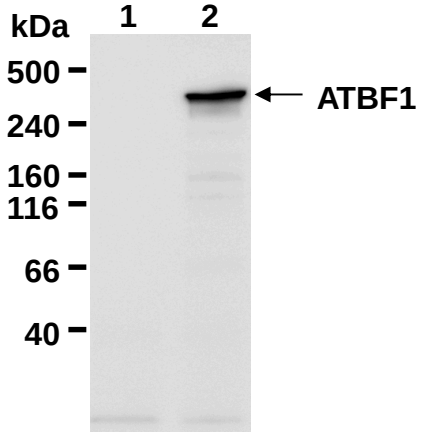
Western blotting analysis of ATBF1 using PD011.
Lane1: mouse embryonal carcinoma cells, undifferentiated P19 (negative control)
Lane2: retinoic acid induced neuronal differentiated P19 (positive control)

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°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°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 1.5 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 20 µL of the sample per lane in 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 in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol) for 1 hour at 12V or in a tank transfer system (25 mM Tris, 190 mM glycine, 10% methanol) for 2 hour at 50V. See the manufacturer'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 for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) milk as suggested in the **APPLICATIONS**. (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).
9) Incubate the membrane with 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.5) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3).
11) Wipe excess buffer off the membrane, and incubate membrane with appropriate 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. Develop the film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of ATBF1 using PD011. *After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with anti-ATBF1 (AT-6) monoclonal antibody.*
Lane1: HA-tag transfected HEK293T
Lane2: HA-tagged ATBF1 transfected HEK293T

This data was provided by Dr. Nan Gao, Dr Tae-Sun Kim, and Dr. Yutaka Miura(Department of Molecular Neurobiology, Nagoya City University, Graduate School of Medical Sciences).

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
3) Mix the Dynabeads protein G well and take 50 µL of beads to the new tube, remove the stocking solution by applying the tube on the magnet.
4) Add primary antibody as suggested in the **APPLICATIONS** and 200 µL of cold Lysis buffer. Mix well and incubate for 10 minutes. Then remove the supernatant by applying the tube on the magnet.
5) Immediately add 300 µg of the cell lysates into the tube, mix well and incubate with gentle agitation for 2 hours at 4°C.
6) Remove the supernatant by applying the tubes on the magnet.
7) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
8) Resuspend the beads with cold Lysis buffer.
9) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
10) Repeat steps 8)-9) 3-5 times.
11) Resuspend the beads in 25 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; transfectant)

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