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Not for use in diagnostic procedures.

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POLYCLONAL ANTIBODY

Anti-ATBF1 (D1-120) pAb

Code No.	Quantity	Form
PD010	100 µL	Rabbit IgG

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. The rabbit was immunized with recombinant region of human ATBF1 (2107-2147 aa.) corresponding to the identical amino acid sequence of mouse ATBF1 (2114-2154 aa.).

FORMULATION: 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 internal region of ATBF1 on Western blotting, Immunoprecipitation, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:2,000
Immunoprecipitation; 1 µL/150 µg of cell lysate
Immunohistochemistry; 1:2,500-5,000 (DAB staining)
1:500 (Immunofluorescence)
Fixation; 4% paraformaldehyde
Heat treatment is necessary for paraffin embedded sections.
Pressure Cooker; 5 minutes at 110°C in 10 mM citrate buffer (pH 6.0)
Immunocytochemistry; 1:500
Flow cytometry; Not tested

Detailed procedures are provided in the following PROTOCOLS.

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

1) Uhm, K. O., *et al., J. Alzheimers Dis.* **43**, 243-257 (2015) [IHC]
2) Zhang, S., *et al*., *Biochem. Biophys. Res. Commu*n. **427**, 537-541 (2012) [WB]
3) Kim, T. S., *et al., Dis. Model Mech.* **3**, 752-762 (2010) [IHC]
4) Jung, C. G., *et al., Development* **132**, 5137-5145 (2005) [IF]
5) Zhang, Z., *et al., Clin. Cancer Res*. **11**, 193-198 (2005)
6) Ishii, Y., *et al., J. Comp. Neurol.* **465**, 57-71 (2003)
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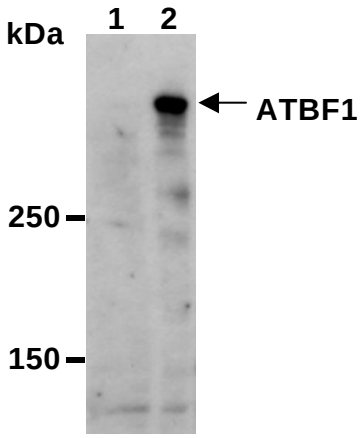
8) Miura, Y., *et al., J. Biol. Chem*. **270**, 26840-26848 (1995)

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Western blot analysis of ATBF1 using PD010.
Lane1: mouse embryonal carcinoma cells, undifferentiated P19 (negative control)
Lane2: retinoic acid induced neuronal differentiated P19 (positive control)

PROTOCOLS:

SDS-PAGE & Western Blotting

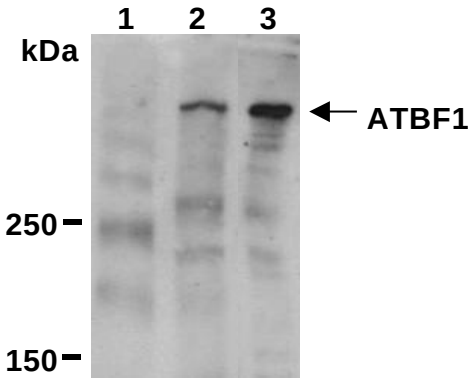
1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4ºC with rotating for 30 minutes; thereafter, briefly sonicate 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% MeOH) for 1 hour at 12V or in a tank transfer system (25 mM Tris, 190 mM glycine, 10% MeOH) 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 TBS (50mM Tris-HCl, pH 7.5, 150 mM NaCl)] 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 TBS (pH 7.5) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with TBS-T [0.05% Tween-20 in TBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in TBS, pH 7.5) for 1 hour at room temperature.
10) Wash the membrane with TBS-T (10 minutes x 3 times).
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 1 minute. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; differentiated P19)

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 7.5), 150 mM NaCl, 0.1% 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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 150 μg of the supernatant. Mix well and incubate with gentle agitation for 60-120 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 60 minutes 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; differentiated P19)



Immunoprecipitation of ATBF1 using PD010. *Retinoic acid induced neuronal differentiated P19 cells lysates were immunoprecipitated with antibody, and the immunocomplexes were resolved on SDS-PAGE and immunoblotted with PD010.*
Lane1: IP with normal rabbit IgG
Lane2: IP with PD010
Lane3: Lysate (positive control).

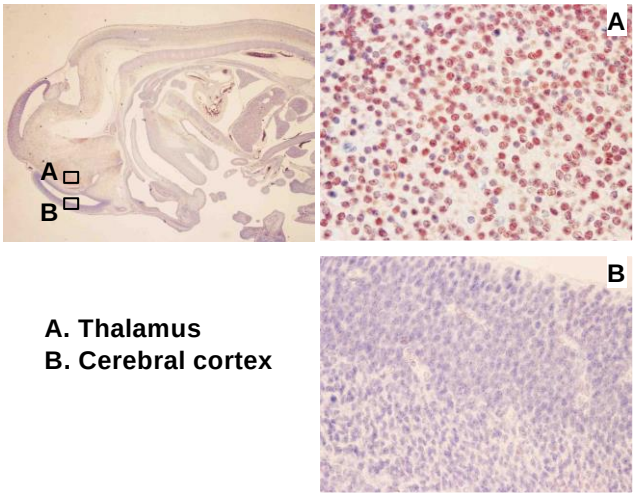
Immunohistochemical staining for paraffin-embedded sections (DAB staining)

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 3 times in PBS for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Pressure cooker:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with aluminum foil. Then put a Pressure cooker on the range. Steam begins to appear, continue it for 5 minutes. Stop the fire and keep it for 5 minutes. Let the pressure out of a cooker and wait for 5 minutes. Take out a beaker and cool it for 20 minutes at room temperature.
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 blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer 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 ENVISION+Dual Link (DAKO; code no. K4063). Incubate for 1 hour at room temperature. Wash as in step 8).
11) Visualize by reacting for 10 minutes with DAB substrate solution (DAKO; code no. K3465). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) 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.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; E14 rat brain)



A. Thalamus
B. Cerebral cortex

Immunohistochemical detection of ATBF1 on paraffin embedded section of E14 rat embryonic brain thalamus and cerebral cortex with PD010.

This data was provided by Dr. Makoto Kawaguchi. (Niigata Rosai Hospital, Japan Labor health and Welfare Organization)

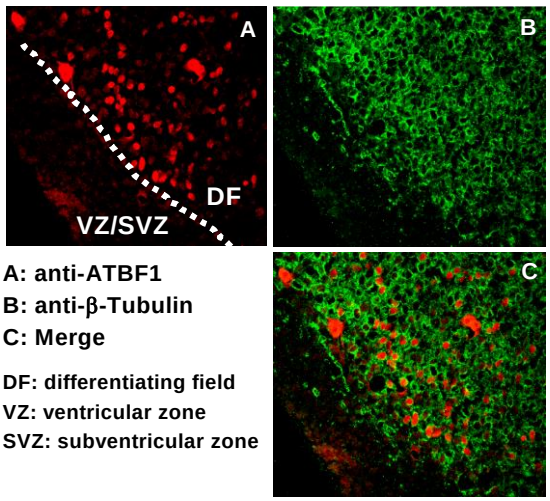
Immunohistochemical staining for paraffin-embedded sections (Immunofluorescence)

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 3 times in PBS for 3-5 minutes each.
4) Heat treatment

   Heat treatment by Pressure cooker:

   Place the slides put on staining basket in 500mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with aluminum foil. Then put a Pressure cooker on the range. Steam begins to appear, continue it for 5 minutes. Stop the fire and keep it for 5 minutes. Let the pressure out of a cooker and wait for 5 minutes. Take out a beaker and cool it for 20 minutes at room temperature.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (3% goat serum, 0.25% Triton X-100 in PBS) for 30 minutes at room temperature to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% goat serum in PBS as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 2 times in PBS for 5 minutes each.
9) Cover tissues with 1:300 Alexa Fluor® 594 conjugated anti-rabbit IgG (Invitrogen) diluted with 1% goat serum in PBS. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
10) Wash the slides 2 times in PBS for 5 minutes each.
11) Wipe excess liquid off the slide but take care not to touch the tissues. Never leave the tissues to dry. Promptly add mounting medium onto the slide, then put a cover slip on it.
12) Now ready for mounting.

(Positive control for Immunohistochemistry; E14 rat brain)



A: anti-ATBF1
B: anti-β-Tubulin
C: Merge

DF: differentiating field
VZ: ventricular zone
SVZ: subventricular zone

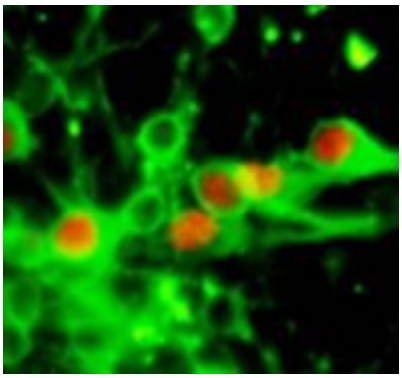
Immunofluorescence detection of ATBF1 on paraffin embedded section of E14 rat embryonic brain with PD010.

This data was provided by Dr. Cha-Gyun Jung. (Research Institute, National Center for Geriatrics and Gerontology)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 cells of transfectant cells for one slide, then incubate in a CO_2 incubator for one night)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.
4) Wash the glass slide 3 times with PBS.
5) Immerse the slide in PBS containing 3% goat serum and 0.2% Triton-X for 30 minutes at room temperature.
6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) Wash the glass slide 3 times with PBS.
8) Add 100 µL of 1:1,000 Alexa Fluor® 594 conjugated anti-rabbit IgG (Invitrogen; A11012) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
9) Wash the glass slide 3 times with PBS.
10) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Rat neuron)



Immunocytochemical detection of ATBF1 in neurons derived from rat neural stem cells with PD010.
Red: anti-ATBF1
Green: anti-β-Tubulin III

This data was provided by Dr. Cha-Gyun Jung. (Research Institute, National Center for Geriatrics and Gerontology)

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