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POLYCLONAL ANTIBODY

Anti-Pax6 pAb

Code No.	Quantity	Form
PD022	100 µL	Affinity Purified

BACKGROUND: Pax6 is a transcription factor that was first identified as a member of paired box (Pax) family protein and functions as the master gene controlling eye development. The mammalian Pax6 locus encodes at least three isoforms, the "canonical" Pax6, Pax6(5a), and Pax6(ΔPD). Pax6 is also involved in various phenomena during development of the central nervous system, including patterning of the neural tube, migration of neurons, and formation of neural circuits. During development, Pax6 is expressed in neuroepithelial cells in the ventricular zone of the brain primordium and regulates proliferation and differentiation through the control of expression of different downstream factors such as FABP7 and Neurogenin2. On the other hand, Pax6 is expressed in neural stem/progenitor cells in the subgaranular zone of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricule throughout adult. Furthermore, intense expression of Pax6 is detected in SVZ-derived immature migrating neurons, cerebellar granule cells, precerebellar neurons and astrocytes in the cerebral cortex and spinal cord.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with synthetic peptide corresponding to C-terminus of mouse Pax6 (420-436 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 Pax6 on Western blotting and Immunohistochemistry. Additionally, it reacts with canonical Pax6 and Pax6(5a) in mouse embryonic cortex on Western blotting.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry (paraffin section); 1:200
Heat treatment: Microwave oven
2 times for 10 minutes each in 10 mM citrate buffer (pH 6.2)
Immunohistochemistry (frozen section); 1:1000
Heat treatment: Microwave oven
1 minute in 10 mM citrate buffer (pH 6.0)
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Chicken
Tissue	Not Tested	whole embryo	whole embryo	whole embryo
Reactivity on IHC		+	+	+

INTENDED USE:
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REFERENCES:
1) Sakurai, K., and Osumi, N., *J. Neurosci.* **28**, 4604-4612 (2008)
2) Osumi, N., *et al., Stem Cells* **26**, 1663-1672 (2008)
3) Nomura, T., *et al*., *PLoS ONE* **3**, e1454 (2008)
4) Maekawa, M., *et al., Genes Cells* **10**, 1001-1114 (2005)
5) Takahashi, M., and Osumi, N., *Development* **129**, 1327-1338 (2002)
6) Matsunaga, E., *et al., Development*, **127**, 2357-2365 (2000)
7) Inoue, T., *et al., Dev Biol*, **219**, 373-383 (2000)

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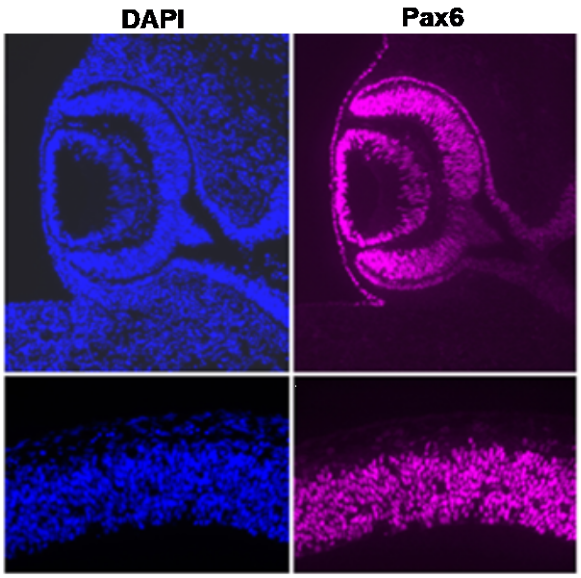
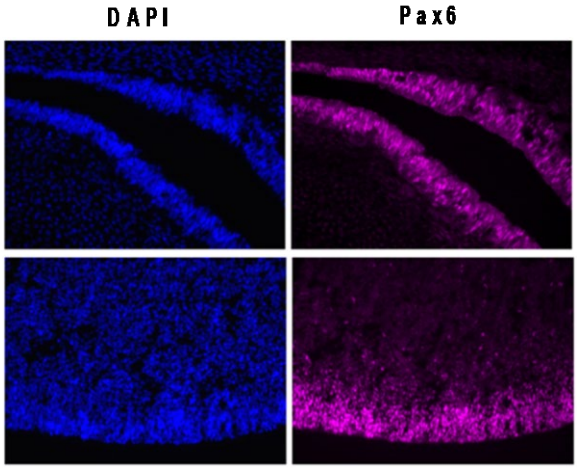
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Immunohistochemical detection of Pax6 on frozen section of SD rat whole embryo at E13.5 with PD022.
(upper panels: eye, lower panels: cerebral cortex)



Immunohistochemical detection of Pax6 on frozen section of chicken whole embryo at E7.0 with PD022.
(upper panels: olfactory epithelium, lower panels: pallium)

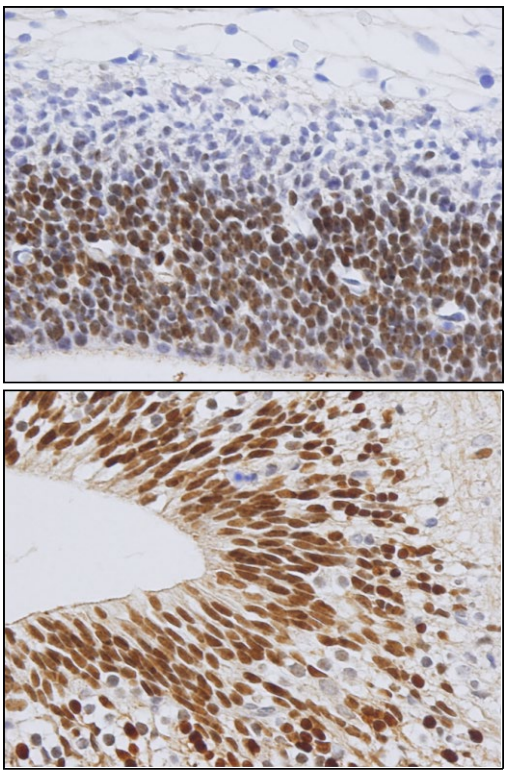
These data were provided by Dr. Takahashi and Dr. Osumi (Division of Developmental Neuroscience, CTAAR, Tohoku University Graduate School of Medicine)

PROTOCOLS:

Immunohistochemical staining for frozen sections

1) Wash the slides with TBS-T (0.1% Tween-20 in TBS) 2 times for 5 minutes each.
2) Heat treatment
   Heat treatment by Microwave:
   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 plastic wrap, then process the slide 1 minute at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 10 minutes. Wash as in step 1).
3) Remove the slides from the TBS-T, wipe gently around each section and cover tissues with blocking buffer (2% goat serum in TBS-T) for 30 minutes to block non-specific staining. Do not wash.
4) 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**.
5) Incubate the sections over night at 4ºC.
6) Wash the slides 3 times in TBS for 5 minutes each.
7) Wipe gently around each section and cover tissues with Cy3 labeled anti-rabbit IgG (Jackson; code no. 711-165-152). Incubate for 1 hour at room temperature. Wash as in step 6).
8) Wipe gently around each section and cover tissues with DAPI in TBS-T. Incubate for 5 minutes.
9) Wash the slides in TBS-T for 5 minutes.
10) Now ready for mounting.

(Positive controls for Immunohistochemistry; SD rat whole embryo at E13.5, chicken whole embryo at E7.0)

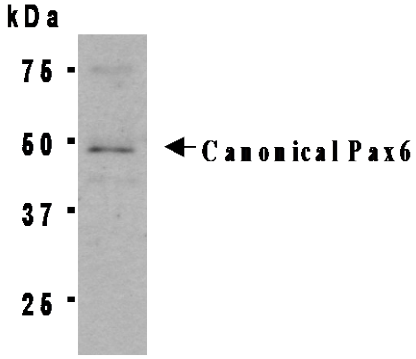


Immunohistochemical detection of Pax6 on paraffin embedded section of mouse whole embryo at E14.5 with PD022.

Immunohistochemical staining for paraffin-embedded sections

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:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.2). 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 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 Histostar™ (Rb) for Mouse tissue (MBL; code no. 8470). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *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; mouse whole embryo at E14.5)



Western blot analysis of Pax6 expression in transfectant using PD022. Cell lysate was provided by Dr. Sakurai and Dr. Osumi (Division of Developmental Neuroscience, CTAAR, Tohoku University Graduate School of Medicine)

SDS-PAGE & Western blotting

1) Wash the $1x10^6$ cells (transfectant) 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 5 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) 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% Methanol). See the manufacture's manual for precise transfer procedure.
4) 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.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 anti-IgG (H+L chain) (Rabbit) pAb (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) 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.
10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.