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Not for use in diagnostic procedures.

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Anti-Autotaxin mAb

CODE No.	D323-3
CLONALITY	Monoclonal
CLONE	4F1
ISOTYPE	Rat IgG2a κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Recombinant Autotaxin (N-terminus)
FORMULATION	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.

APPLICATION-CONFIRMED

Immunohistochemistry 5 µg/mL
Heat treatment for paraffin embedded section:
Autoclave; 110°C for 5 minutes in 10 mM citrate buffer (pH 6.0)

APPLICATION-REPORTED

Western blotting Reference 1) and 2)

SPECIES CROSS REACTIVITY on IHC

Species	Human	Mouse	Rat	Hamster
Cell	Reference 2)	Brain	Not tested	Not tested
Reactivity	+	+	Not tested	Not tested

Entrez Gene ID 5168 (Human), 18606 (Mouse)

REFERENCES

1) Nikitopoulou, I., *et al.*, *J. Exp. Med.* **209**, 925-933 (2012) [WB, IHC-P]
2) Nouh, M. A., *et al.*, *Cancer Sci.* **100**, 1631-1638 (2009) [WB, IHC-P]
3) Tanaka, M., *et al.*, *FEBS Lett.* **571**, 197-204 (2004)
4) Hashimoto, T., *et al.*, *J. Biochem.* **151**, 89-97 (2012)
5) Kishi, Y., *et al.*, *J. Biol. Chem.* **281**, 17492-17500 (2006)

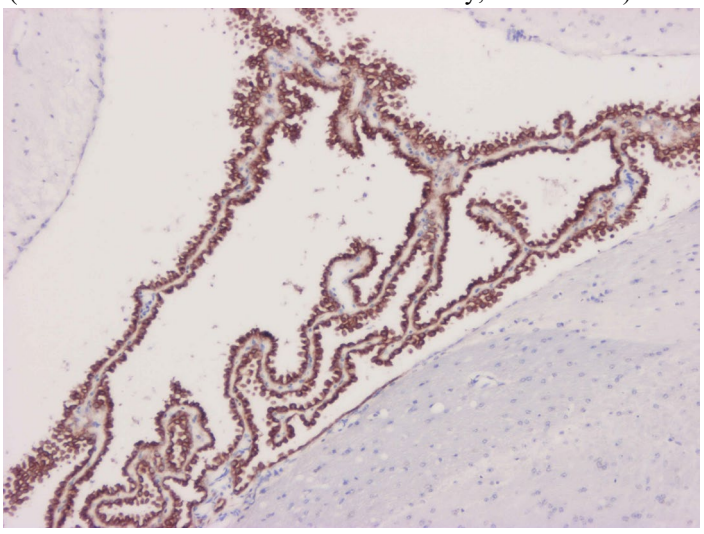
For more information, please visit our web site **http://ruo.mbl.co.jp/.**

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

Immunohistochemical staining for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 3 min. each.
2) Wash the slides with Ethanol 3 times for 3 min. each.
3) Wash the slides with PBS 3 times for 3 min. each.
4) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH6.0) for 5 min. at 110°C using autoclave.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Wash the slides with running water for 5 min., then wash with TBS for 5 min.
7) Remove the slides from TBS and block endogenous peroxidase with 3% H_2O_2 in distilled water for 20 min.
8) Wash the slides with running water for 5 min., then wash with TBS for 5 min.
9) Remove the slides from TBS, wipe gently around each section and cover tissues with blocking buffer (10% normal rabbit serum /PBS) for 30 min. at room temperature (20~25°C) to block non-specific staining. Do not wash.
10) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% BSA/PBS as suggested in the **APPLICATIONS** for overnight at 4°C. (The concentration of antibody will depend on the conditions.)
11) Wash the slides 3 times in TBS for 5 min. each.
12) Wipe gently around each section and cover tissues with Histostar (Rat) (MBL; code no. 8463). Incubate for 30 min. at room temperature.
13) Wash the slides 3 times in TBS for 5 min. each.
14) Visualize by reacting for 5 min. with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Wash the slides in water for 5 min.
16) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
17) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; mouse brain)



Immunohistochemical detection of mouse Autotaxin in mouse brain
Brown: D323-3
Blue: Hematoxylin