For Research Use Only.
Not for use in diagnostic procedures.

Anti-Autotaxin mAb

CODE No. D322-3

CLONALITY Monoclonal
CLONE 3D1
ISOTYPE Rat IgG1 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
Western blotting 1 µg/mL for chemiluminescence detection system

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Samples	Reference 2) and 3)	Supernatant of transfectant Brain	Not tested	Not tested
Reactivity	+	+	Not tested	Not tested

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

REFERENCES
1) Hashimoto, T., *et al.*, *J. Biochem.* **151**, 89-97 (2012)
2) Tanaka, M., *et al.*, *FEBS Lett*, **571**, 197-204 (2004) [WB]
3) Kishi, Y., *et al.*, *J. Biol. Chem.*, **281**, 17492-17500 (2006) [WB]
4) Pamuklar, Z., *et al.*, *J. Biol. Chem.*, **284**, 7385-7394 (2009) [WB]
5) Nikitopoulou, I., *et al.*, *J. Exp. Med.* **209**, 925-933 (2012)

RELATED PRODUCTS
D322-3 Anti-Autotaxin mAb (3D1)
D323-3 Anti-Autotaxin mAb (4F1)

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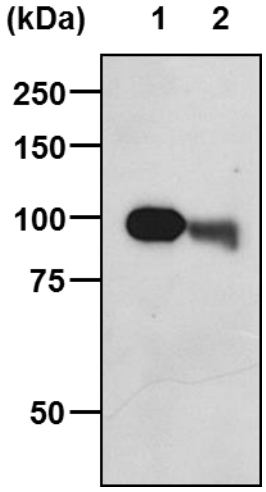
SDS-PAGE & Western blotting

1) Mix the culure supernatant of Autotaxin expressed transfectant or mouse brain with 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge at 12,000 xg for 5min. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer'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 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T (5 min. x 3 times)
7) Incubate the membrane with HRP-conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times)
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. 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 min. Develop the film as usual. The condition for exposure and development may vary.



Western blot analysis of Autotaxin

Lane 1: Culture supernatant of Autotaxin transfectant (10 μL/lane)
Lane 2: Mouse brain lysate (25 ng/lane)

Immunoblotted with D322-3