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

Anti-GNAT2 (Zebrafish) pAb

CODE No. PM075

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 μL

SOURCE Purified IgG from rabbit serum
IMMUNOGEN KLH conjugated synthetic peptide, MDRICKPDYLPT (corresponding to amino acid residues 159-170 of zebrafish gnat2)
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.

APPLICATIONS-CONFIRMED

Western blotting	1:1,000-2,000 for chemiluminescence detection system
Immunofluorescence	1:800 (frozen section)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Zebrafish
Cell	Not tested	Not tested	Not tested	Adult zebrafish retina
Reactivity				+

Entrez Gene ID 140429 (Zebrafish)

REFERENCES

1) Corral-Serrano, J. C., *et al., Sci. Rep*. **8**, 9675 (2018) [IF]
2) Yu, M., *et al*., *Biol. Open* **5**, 1662-1673 (2016) [WB, IF]
3) Ogawa, Y., *et al*., *Proc. Biol. Sci.* **282**, 20150659 (2015) [IF]
4) Brockerhoff, S. E., *et al*., *J. Neurosci.* **23**, 470-480 (2003)

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RELATED PRODUCTS

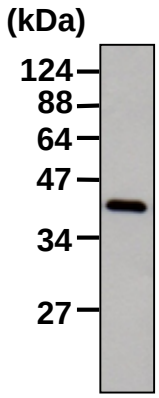
Antibodies

PM092	Anti-NR1D1 (Rev-erbα) pAb
PM093	Anti-NR1D2 (Rev-erbβ) pAb
PM087	Anti-Chrono (Mouse) pAb
D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D349-3	Anti-CLOCK (Mouse) mAb (CLSP4)
D335-3	Anti-BMAL1 mAb (B1BH2)
D361-3	Anti-BMAL1 mAb (2F11)
M225-3	Anti-NFIL3 (E4BP4) chimeric mAb (42)
PM097	Anti-NFIL3 (E4BP4) pAb
PM079	Anti-DBP (Mouse) pAb
PM081	Anti-Cry1 (Mouse) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM091	Anti-Per1 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
PM096	Anti-PER2 (Human) pAb
M219-3	Anti-RORγt mAb (4H11)
PM080	Anti-RORγt pAb

SDS-PAGE & Western blotting

1) Boil the sample for 5 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
2) 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.
3) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
4) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) 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.

(Positive control for Western blotting; Adult zebrafish retina)



Western blot analysis of GNAT2 from adult zebrafish retina

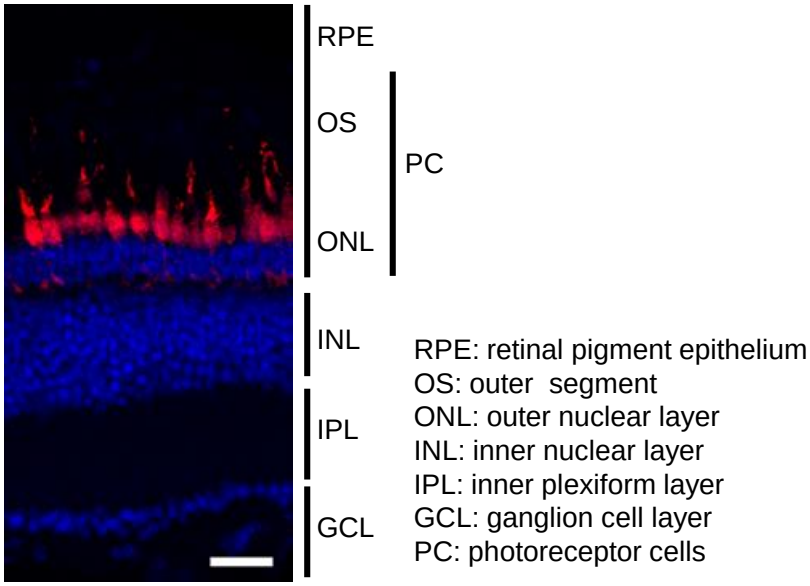
Immunoblotted with Anti-GNAT2 (Zebrafish) pAb (PM075)

This sample was kindly provided by Drs. Tomoya Shiraki, Daisuke Kojima, and Yoshitaka Fukada. (Department of Biophysics and Biochemistry, Graduate school of Science, The University of Tokyo)

Immunofluorescence

1) Wash the slides with PBS 3 times.
2) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (3% goat serum/0.1% Triton X-100/PBS) for 1 hr. at room temperature to block non-specific staining. Do not wash.
3) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggest in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.) Incubate the sections overnight at 4°C.
4) Wash the slides with PBS (10 min. x 3 times).
5) Wipe gently around each section and cover tissues with 1:1,000 of Alexa Fluor® 568 Goat Anti-Rabbit IgG (Molecular Probes; code no. A11036). Incubate for 4 hr. at room temperature.
6) Wash the slides 3 times in PBS (10 min. x 3 times).
7) 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.
8) Now ready for mounting.

(Positive control for Immunofluorescence; Adult zebrafish retina)



Scale bar = 25 μm

Immunofluorescent detection of GNAT2 on adult zebrafish retina

Red: Anti-GNAT2 (Zebrafish) pAb (PM075)
Blue: DAPI

Data were provided by Drs. Tomoya Shiraki, Daisuke Kojima, and Yoshitaka Fukada. (Department of Biophysics and Biochemistry, Graduate school of Science, The University of Tokyo)