

MONOCLONAL ANTIBODY

Rat IgG2a (isotype control)-Agarose

Code No.	Clone	Quantity
M081-8	2H3	Gel: 200 μ L

SOURCE: This antibody was purified from hybridoma (clone 2H3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Rat lymphocyte nodes immunized with KLH.

FORMULATION: 100 μ g of Rat IgG2a covalently coupled to 200 μ L of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.09% sodium azide) for a total volume of 400 μ L.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: No specific binding was detected on Immunoprecipitation.

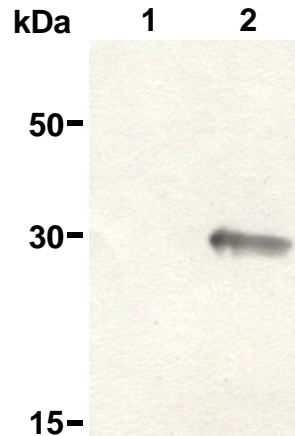
APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; 20 μ L of gel slurry
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; Not tested

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

INTENDED USE:

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



Immunoprecipitation of GFP fused protein from transfectant with M081-8 (1) or Agarose-anti-GFP monoclonal antibody (MBL; code no. D153-8) (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with Anti-GFP monoclonal antibody (MBL; code no. M048-3).

PROTOCOL:

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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.
- 3) Add agarose as suggest in the **APPLICATIONS** into 200 μ L of cell extract. Mix well and incubate with gentle agitation for 30-120 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 agarose in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 6) Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour 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.
- 8) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room

temperature, or overnight at 4°C.

- 9) Incubate the membrane with the 1 µg/mL anti-GFP monoclonal antibody (MBL; code no. M048-3) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 11) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 12) Wash the membrane with PBS-T (5 minutes x 3 times).
- 13) 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.
- 14) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

M081-5	Rat IgG2a isotype control-PE (2H3)
M081-8	Rat IgG2a isotype control-Agarose (2H3)
M081-A48	Rat IgG2a isotype control-Alexa Fluor [®] 488 (2H3)
M081-A64	Rat IgG2a isotype control-Alexa Fluor [®] 647 (2H3)
M090-3	Rat IgG2b isotype control (3G8)
M090-4	Rat IgG2b isotype control-FITC (3G8)
M090-5	Rat IgG2b isotype control-PE (3G8)
M090-A48	Rat IgG2b isotype control-Alexa Fluor [®] 488 (3G8)
M090-A64	Rat IgG2b isotype control-Alexa Fluor [®] 647 (3G8)
M082-3	Rat IgG2c isotype control (6E12)
M082-4	Rat IgG2c isotype control-FITC (6E12)
M189-3	Hamster IgG isotype control (tkko)
PM035	Normal Rabbit IgG (polyclonal)
PM035-8	Normal Rabbit IgG-Agarose (polyclonal)
PM067	Normal Guinea Pig IgG (polyclonal)

RELATED PRODUCTS:

Functional grade antibodies

M075-3M2	Mouse IgG1 isotype control FG (2E12)
M076-3M2	Mouse IgG2a isotype control FG (6H3)
M077-3M2	Mouse IgG2b isotype control FG (3D12)
M077-3M2	Mouse IgG3 isotype control FG (6A3)
M080-3M2	Rat IgG1 isotype control FG (1H5)
M081-3M2	Rat IgG2a isotype control FG (2H3)
M090-3M2	Rat IgG2b isotype control FG (3G8)
M082-3M2	Rat IgG2c isotype control FG (6E12)

Purified antibodies

M075-3	Mouse IgG1 isotype control (2E12)
M075-4	Mouse IgG1 isotype control-FITC (2E12)
M075-5	Mouse IgG1 isotype control-PE (2E12)
M075-8	Mouse IgG1 isotype control-Agarose (2E12)
M075-A48	Mouse IgG1 isotype control-Alexa Fluor [®] 488 (2E12)
M075-A64	Mouse IgG1 isotype control-Alexa Fluor [®] 647 (2E12)
M076-3	Mouse IgG2a isotype control (6H3)
M076-4	Mouse IgG2a isotype control-FITC (6H3)
M076-5	Mouse IgG2a isotype control-PE (6H3)
M076-A48	Mouse IgG2a isotype control-Alexa Fluor [®] 488 (6H3)
M076-A64	Mouse IgG2a isotype control-Alexa Fluor [®] 647 (6H3)
M077-3	Mouse IgG2b isotype control (3D12)
M077-4	Mouse IgG2b isotype control-FITC (3D12)
M077-5	Mouse IgG2b isotype control-PE (3D12)
M077-A48	Mouse IgG2b isotype control-Alexa Fluor [®] 488 (3D12)
M077-A64	Mouse IgG2b isotype control-Alexa Fluor [®] 647 (3D12)
M078-3	Mouse IgG3 isotype control (6A3)
M078-4	Mouse IgG3 isotype control-FITC (6A3)
M079-3	Mouse IgM isotype control (7E10)
M080-3	Rat IgG1 isotype control (1H5)
M080-4	Rat IgG1 isotype control-FITC (1H5)
M080-5	Rat IgG1 isotype control-PE (1H5)
M080-A48	Rat IgG1 isotype control-Alexa Fluor [®] 488 (1H5)
M080-A64	Rat IgG1 isotype control-Alexa Fluor [®] 647 (1H5)
M081-3	Rat IgG2a isotype control (2H3)
M081-4	Rat IgG2a isotype control-FITC (2H3)