

MONOCLONAL ANTIBODY

Loading Control Antibody

Anti-GAPDH mAb

Code No.	Clone	Subclass	Quantity	Concentration
M171-3	3H12	Mouse IgG2a κ	100 μ L	3 mg/mL

BACKGROUND: GAPDH (Glyceraldehyde-3-phosphate Dehydrogenase) is a well-known enzyme, which catalyzes of glycolysis. GAPDH is stably and constitutively expressed at high levels in most tissues and cells, it is considered a housekeeping protein. Therefore, GAPDH is often used as a loading control of WB.

SOURCE: This antibody was purified from hybridoma (clone 3H12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the GAPDH from rabbit muscle.

FORMULATION: 300 μ g IgG in 100 μ L 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 GAPDH on Western blotting.

APPLICATIONS:

Western blotting; 3 μ g/mL for chemiluminescence detection system

Immunoprecipitation; Not recommended

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster	Chicken	Monkey
Cells	HeLa	NIH/3T3	PC12	CHO	MuH1	COS-7
Reactivity on WB	+	+	+	+	+	+*

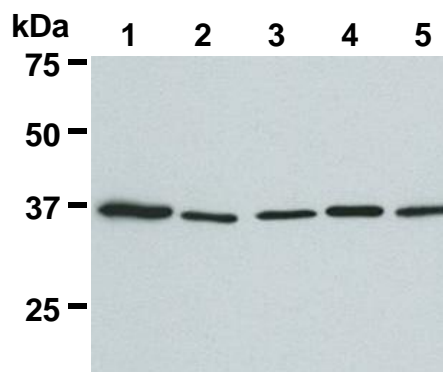
*Reactivity of clone 3H12 to monkey is not confirmed in our laboratory. However, it is reported that this clone reacts with COS-7 cells⁴⁾.

INTENDED USE:

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

REFERENCES:

- 1) Mori, T., *et al.*, *Sci. Rep.* **8**, 1294 (2018) [WB]
- 2) Huang, Y., *et al.*, *Oncotarget* **8**, 83075-83087 (2017) [WB]
- 3) Ju., J., *et al.*, *Nat. Commun.* **8**, 928 (2017) [WB]
- 4) Fu, K., *et al.*, *Sci. Rep.* **7**, 13084 (2017) [WB]
- 5) Chen, L., *et al.*, *Oncotarget* **8**, 63825-63834 (2017) [WB]
- 6) Li, L., *et al.*, *Nat. Commun.* **8**, 691 (2017) [WB]
- 7) Wang, S., *et al.*, *Cell Death Dis.* **8**, e3058 (2017) [WB]
- 8) Lv, J., *et al.*, *Mol. Med. Rep.* **16**, 4475-4482 (2017) [WB]
- 9) Xu, J., *et al.*, *PNAS.* **114**, 8620-8625 (2017) [WB]
- 10) Uemura, Y., *et al.*, *Genes Cells* **22**, 785-798 (2017) [WB]
- 11) Gao, Y., *et al.*, *Oncotarget.* **8**, 7420-7440 (2017) [WB]
- 12) Miyake, K., *et al.*, *Cell Rep.* **17**, 2004-2014 (2016) [WB]
- 13) Yoshimoto., R., *et al.*, *RNA* **23**, 47-57 (2017) [WB]
- 14) Mita., T., *et al.*, *J. Biol. Chem.* **291**, 4955-4965 (2016) [WB]
- 15) Liu, S., *et al.*, *Exp. Ther. Med.* **9**, 1597-1604 (2015) [WB]
- 16) Li, Q *et al.*, *Nat. Commun.* **6**, 6183 (2015) [WB]
- 17) Tarze, A., *et al.*, *Oncogene* **26**, 2606-2620 (2007)
- 18) Barber, R. D., *et al.*, *Physiol. Genomics.* **21**, 389-395 (2005)



Western blot analysis of GAPDH expression on HeLa (1), NIH/3T3 (2), PC12 (3), CHO (4) and MuH1 (5) using M171-3.

Sample volume: 2 μ g/lane

PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash cells (approximately 1×10^7 cells) 3 times with PBS and resuspend them in 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% NP-40, 2 mM EDTA, 10% glycerol) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 0.4 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 5) 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.
- 6) To reduce nonspecific binding, soak the membrane in 7.5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (5 minutes x 3 times).
- 11) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 13) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; HeLa, NIH/3T3, PC12, CHO and MuH1)

RELATED PRODUCTS:

Loading control antibody

M171-7	Anti-GAPDH mAb-HRP-DirecT (3H12)
M177-3	Anti-β-Actin mAb (6D1)
PM053	Anti-β-Actin pAb (polyclonal)
PM053-7	Anti-β-Actin pAb-HRP-DirecT (polyclonal)
M175-3	Anti-α-Tubulin mAb (2F9)
PM054	Anti-α-Tubulin pAb (polyclonal)
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT (polyclonal)
PM064	Anti-Lamin B1 pAb (polyclonal)
PM088	Anti-Vinculin pAb (polyclonal)

Isotype control

M076-3	Mouse IgG2a (isotype control) (6H3)
M076-3M2	Mouse IgG2a (isotype control) FG (6H3)
M076-4	Mouse IgG2a (isotype control)-FITC (6H3)
M076-5	Mouse IgG2a (isotype control)-PE (6H3)
M076-6	Mouse IgG2a (isotype control)-Biotin (6H3)

M076-11	Mouse IgG2a (isotype control)-Magnetic Beads (6H3)
M076-12	Mouse IgG2a (isotype control)-ALP (6H3)
M076-A48	Mouse IgG2a (isotype control)-Alexa Fluor [®] 488 (6H3)
M076-A64	Mouse IgG2a (isotype control)-Alexa Fluor [®] 647 (6H3)
M075-3	Mouse IgG1 (isotype control) (2E12)
M075-3M2	Mouse IgG1 (isotype control) FG (2E12)
M075-4	Mouse IgG1 (isotype control)-FITC (2E12)
M075-5	Mouse IgG1 (isotype control)-PE (2E12)
M075-6	Mouse IgG1 (isotype control)-Biotin (2E12)
M075-8	Mouse IgG1 (isotype control)-Agarose (2E12)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads (2E12)
M075-12	Mouse IgG1 (isotype control)-ALP (2E12)
M075-A48	Mouse IgG1 (isotype control)-Alexa Fluor [®] 488 (2E12)
M075-A64	Mouse IgG1 (isotype control)-Alexa Fluor [®] 647 (2E12)
M077-3	Mouse IgG2b (isotype control) (3D12)
M077-3M2	Mouse IgG2b (isotype control) FG (3D12)
M077-4	Mouse IgG2b (isotype control)-FITC (3D12)
M077-5	Mouse IgG2b (isotype control)-PE (3D12)
M077-6	Mouse IgG2b (isotype control)-Biotin (3D12)
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads (3D12)
M077-12	Mouse IgG2b (isotype control)-ALP (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)
M078-3M2	Mouse IgG3 (isotype control) (6A3)
M078-4	Mouse IgG3 (isotype control)-FITC (6A3)
M078-6	Mouse IgG3 (isotype control)-Biotin (6A3)
M079-3	Mouse IgM (isotype control) (7E10)
M080-3	Rat IgG1 (isotype control) (1H5)
M080-3M2	Rat IgG1 (isotype control) FG (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)
M081-3M2	Rat IgG2a (isotype control) FG (2H3)
M081-4	Rat IgG2a (isotype control)-FITC (2H3)
M081-5	Rat IgG2a (isotype control)-PE (2H3)
M081-8	Rat IgG2a (isotype control)-Agarose (2H3)
M081-11	Rat IgG2a (isotype control)-Magnetic Beads (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)
M090-3M2	Rat IgG2b (isotype control) FG (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)
M082-3M2	Rat IgG2c (isotype control) FG (6E12)
M082-4	Rat IgG2c (isotype control)-FITC (6E12)
M189-3	Syrian Hamster IgG (isotype control)
M199-3	Armenian Hamster IgG (isotype control)
PM035	Normal Rabbit IgG (polyclonal)
PM035-8	Normal Rabbit IgG-Agarose (polyclonal)
PM067	Normal Guinea Pig IgG (polyclonal)
PM089	Normal Sheep IgG (polyclonal)
PM094	Normal Goat IgG (polyclonal)