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MONOCLONAL ANTIBODY **Loading Control Antibody**

Anti-GAPDH mAb

Code No.	Clone	Subclass	Quantity	Concentration
M171-3	3H12	Mouse IgG2a κ	100 µL	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 an assay control.

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 µg IgG in 100 µL 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 µg/mL
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

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 cells[4].

INTENDED USE:

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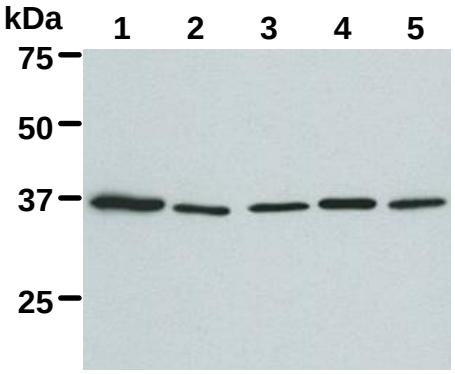
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Western blot analysis of GAPDH expression in HeLa (1), NIH/3T3 (2), PC12 (3), CHO (4) and MuH1 (5) using M171-3.
Sample volume: 2 µg/lane

PROTOCOL:

SDS-PAGE & Western blotting

1) Wash cells (approximately 1 x 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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
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).
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, MuH1)

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