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

MONOCLONAL ANTIBODY

CD29/β1-Integrin

Code No.	Clone	Subclass	Quantity	Form
D050-3	AG89	Mouse IgG1	100 μg	Lyophilized

BACKGROUND: The integrin family of adhesion molecules participate in important cell-cell and cell-extracellular matrix interactions in a diverse range of biological processes. Integrins are heterodimers consisting of a α subunit and β subunit. Both α and β subunits are transmembrane proteins with large extracellular domains (>100 kDa for α subunit and >75 kDa for β subunit) that interact with extracellular matrix proteins and relatively small cytoplasmic domains (50 amino acids or less, except for the β4 subunit) that interact with cytoskeletal proteins. The adhesiveness of integrins is dynamically regulated in response to cytoplasmic signals, termed "inside-out" signaling. It has been reported that, upon ligand binding, integrins regulate many intracellular signaling pathways that involve cytoplasmic alkalization, intracellular Ca^{2+} fluctuation, inositol lipid metabolism, protein kinase C, MAP kinase and phosphatidyl inositol kinase. Anti-integrin monoclonal antibody, AG89, reacts with CD29/integrin β1 chain regardless of the subunit. AG89 can recognize resting site β1 integrin on the cells, but the reactivity is increased ~2-fold upon integrin activation by anti-activating β1 antibodies and ~3-fold by Mn^{2+}. Furthermore, occupation of the ligand-binding pocket by a soluble ligand (RGD peptide for α5β1 and CS-1 peptide for α4β1) resulted in maximum binding of AG89. The epitope for AG89 lies within residues 426-587.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with G-361 human melanoma cell line.

FORMULATION: This antibody is lyophilized form. Prepare a stock solution by dissolving the lyophilized antibody in 100 μL of distilled water. After reconstitution, the IgG concentration should be 1 mg/mL in PBS (pH 7.2)/ 1% sucrose. No preservative contained.

STORAGE: This antibody is stable for one year from the date of shipment when stored at 4°C. After reconstitution, avoid repeated freezing and thawing. For storage, prepare appropriate aliquots and freeze them at -20°C.

REACTIVITY: This antibody reacts with human β1-Integrin on Flow cytometry.

INTENDED USE:
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APPLICATIONS:
Western blotting; 10 μg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
It is reported that this monoclonal antibody can be used in Immunoprecipitation in the reference number 4).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 μg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	MOLT-4, Jurkat	Not Tested	Not Tested
Reactivity on FCM	+		

REFERENCES:
1) Nishiuchi. R., *et al*. *PNAS* **102,** 1939-1944 (2005)
2) Shi, Q., *et al. Mol. Biol. Cell* **14,** 4306-4315 (2003)
3) García, J. A., *et al. J. Biol. Chem.* **273,** 34710-34715 (1998)
4) Tsuchida. J., *et al*. *J. Cell Sci*. **111,** 1759-1766 (1998)
5) Takagi. J., *et al*. *J. Biochem*. **121,** 914-921 (1997)
6) O'Toole, T.E., *et al*. *J. Cell Biol.* **124,** 1047-1059 (1994)

Clone AG89 is used in the reference number 1) - 5).

RELATED PRODUCTS:

D050-5	PE labeled CD29/β1-Integrin (AG89)
K0099-3	Anti-MIBP (5B4.7)
D202-3	mouse CD11b (1C4)
D202-4	FITC labeled mouse CD11b (1C4)
M100-3	mouse CD11c (223H7)
M100-4	FITC labeled mouse CD11c (223H7)
M100-6	Biotin labeled mouse CD11c (223H7)
M109-3	mouse CD61 (1-55-4)
K0046-3	Anti-mouse Integrin α7 (3C12)
K0046-5	PE labeled anti-mouse Integrin α7 (3C12)
K0047-3	Anti-mouse Integrin α7 (6A11)

PROTOCOLS:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2,

250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).

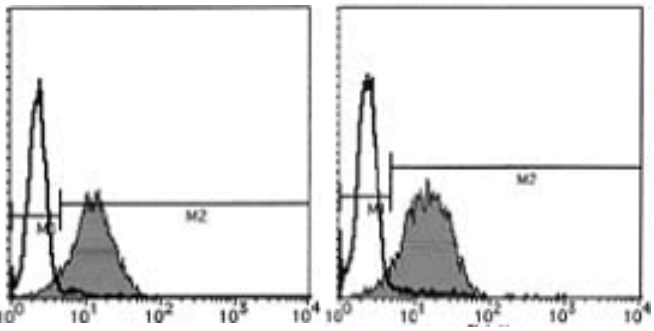
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) 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.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

<u>Flow cytometric analysis for floating cells</u>

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer (5x10^6 cells/mL).
3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 30 μL of primary antibody diluted as suggest in the **APPLICATIONS** to cell pellet and gently mix then incubate for 30 minutes at room temperature (The concentration of antibody will depend on condition).
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; MOLT-4, Jurkat)



Flow cytometric analysis of CD29/β1 Integrin expression on MOLT-4 (left) and Jurkat (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D050-3 to the cells.*