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

MONOCLONAL ANTIBODY

Anti-CD29 (Integrin β1) (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
D050-5	AG89	Mouse IgG1	1 mL (50 tests)

BACKGROUND: The integrin family of adhesion molecules participate in important cell-cell and celle-xtracellular matrix interactions in a diverse range of biological processes. Integrins are heterodimers consisting of a α subunit and β subunit. Both α and β subunit 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 human integrin β1 chain regardless of the a 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 splenocyte immunized with G-361 Human melanoma cell line.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN_3.
*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 solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with human CD29 (Integrin β1) on Flow cytometry.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry ; Not tested
Flow cytometry; 20 μL (ready for use)

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

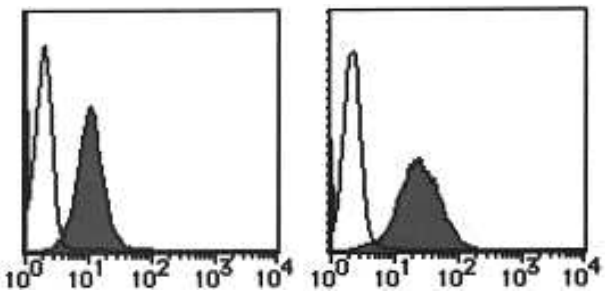
INTENDED USE:
For research use only. Not for clinical diagnosis.

SPECIES CROSS REACTIVITY:

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

REFERENCES:
1) Matsunaga, T., *et al., Ann. Hematol.* **91**, 1633-1643 (2012) [FCM]
2) Nishiuchi, R., *et al. PNAS* **102,** 1939-1944 (2005)
3) Takahashi, H., *et al. J. Biol. Chem.* **275,** 23589-23595 (2002)
4) Tsuchida, J., *et al. J. Cell Sci.* **111,** 1759-1755 (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)

RELATED PRODUCTS:
D050-3 Anti-CD29 (Integrin β1) (Human) mAb (AG89)
M075-5 Mouse IgG1 (isotype control)-PE (2E12)



Flow cytometric analysis of Human β1-Integrin expression on MOLT-4 cells (left) and Jurkat cells (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D050-5 to the cells.*

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PROTOCOL:

Flow cytometric analysis for floating cells

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 0.09% NaN_3].
2) Resuspend the cells with washing buffer (5 x 10^6 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 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 10 minutes at room temperature.
5) Add the primary antibody as suggested in the **APPLICATIONS**. Mix well and incubate for 30 minutes at room temperature.
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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

   (Positive controls for Flow cytometry: MOLT-4 and Jurkat)