

MONOCLONAL ANTIBODY

Anti-Integrin $\alpha 7$ (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0046-3	3C12	Mouse IgG1 κ	100 μ L	1 mg/mL

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 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 $\beta 4$ 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 phosphatidylinositol kinase. Integrin $\alpha 7$ is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms-2 and -4. The $\alpha 7$ subunit is expressed mainly in skeletal and cardiac muscle and may be involved in differentiation and migration processes during myogenesis. Absence of integrin $\alpha 7$ results in muscular dystrophy is revealed.

SOURCE: This antibody was purified from hybridoma using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Integrin $\alpha 7$ knockout C57/B6 mouse splenocyte immunized with mouse myoblasts.

FORMULATION: 100 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 mouse Integrin $\alpha 7$ on Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested	C2C12	Not tested
Reactivity on FCM		+	

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; 10 μ g/mL
- Flow cytometry; 10-20 μ g/mL (final concentration)

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

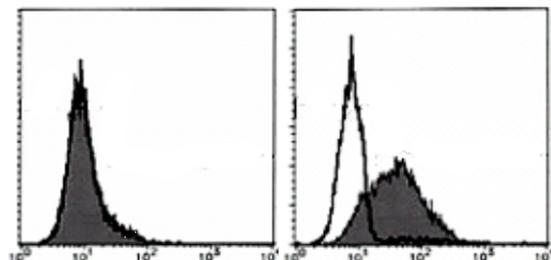
INTENDED USE:

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

REFERENCES:

- 1) Wang, M., *et al.*, *Mol. Cell Biol.* **33**, 678-687 (2013) [FCM]
- 2) Samson, T., *et al.*, *J. Biol. Chem.* **279**, 28641-28652 (2004)
- 3) Volpers, C., *et al.*, *J. Virol.* **77**, 2093-2104 (2003)
- 4) Rosbottom, A., *et al.*, *J. Immunol.* **169**, 5689-5695 (2002)
- 5) von der Mark, H., *et al.*, *J. Biol. Chem.* **277**, 6012-6016 (2002)
- 6) Mielenz, D., *et al.*, *J. Biol. chem.* **276**, 13417-13426 (2001)

Clone 3C12 is used in these references.



Flow cytometric analysis of mouse Integrin $\alpha 7$ expression on NIH/3T3 (left) and C2C12 (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0046-3 to the cells.

PROTOCOLS:

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all step after 2).

- 1) Detach the cells from culture dish by using cell dissociation buffer (Invitrogen; code no. 13151-014).
- 2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) 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.

- 3) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 4) Add 50 μ L 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.
- 5) Add 10 μ L of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 6) Add 40 μ L of the primary antibody at the concentration of as suggest in **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 7) 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.
- 8) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 9) 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.
- 10) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; C2C12)

- D281-A48 Anti-CD61 (GPIIIa) (Human) mAb
-Alexa Fluor[®] 488 (T74)
- D281-A64 Anti-CD61 (GPIIIa) (Human) mAb
-Alexa Fluor[®] 647 (T74)
- M075-3 Mouse IgG1 (isotype control) (2E12)

Immunocytochemistry

- 1) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 2) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
- 3) Add FITC conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 4) Wash the slide in a plenty of PBS as in the step 2).
- 5) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 6) Promptly add mounting medium onto the slide, then put a cover slip on it.

RELATED PRODUCTS:

- K0046-4 Anti-Integrin ± 7 (Mouse) mAb-FITC (3C12)
- K0046-5 Anti-Integrin ± 7 (Mouse) mAb-PE (3C12)
- K0047-3 Anti-Integrin ± 7 (Mouse) mAb (6A11)
- D050-3 Anti-CD29 (Integrin ² 1) (Human) mAb (AG89)
- D050-5 Anti-CD29 (Integrin ² 1) (Human) mAb-PE (AG89)
- D281-3 Anti-CD61 (GPIIIa) (Human) mAb (T74)