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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Integrin α7 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0046-3	3C12	Mouse IgG1 κ	100 µL	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 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. Integrin α7 is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms-2 and -4. The α7 subunit is expressed mainly in skeletal and cardiac muscle and may be involved in differentiation and migration processes during myogenesis. Absence of integrin α7 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 α7 knockout C57/B6 mouse splenocyte immunized with mouse myoblasts.

FORMULATION: 100 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 mouse Integrin α7 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 µg/mL
Flow cytometry; 10-20 µg/mL (final concentration)

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

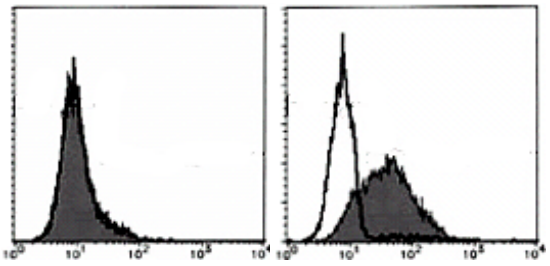
INTENDED USE:

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 α7 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.*

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

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 (Thermo Fisher Scientific, 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 ($5x10^6$ cells/mL).
4) 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.
5) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature normal goat serum containing 1 mg/mL normal human IgG.
6) Add 40 µL of the primary antibody at the concentration of as suggested 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; C2C12)

Immunocytochemistry

1) Add the primary antibody diluted with PBS as suggested 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 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:

Please visit our website at https://ruo.mbl.co.jp/.