

Product datasheet for **AM20011AF-N**

Integrin alpha-7 / ITGA7 Mouse Monoclonal Antibody [Clone ID: 3C12]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3C12
Applications:	FC, IF
Recommended Dilution:	Flow Cytometry: 10-20 µg/ml (final concentration). Immunocytochemistry: 10 µg/ml. <i>Positive Control:</i> C2C12. Detailed Procedure is provided in the following Protocols .
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Mouse myoblasts.
Specificity:	This antibody reacts with Mouse Integrin alpha-7.
Formulation:	PBS, pH 7.2 containing 50% Glycerol without preservatives. State: Azide Free State: Liquid purified IgG fraction.
Concentration:	lot specific
Purification:	Protein-A Agarose Chromatography.
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Database Link:	Q61738



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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 an alpha subunit and a beta subunit. Both alpha and beta subunits are transmembrane proteins with large extracellular domains (>100 kDa for alpha subunit and >75 kDa for beta 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 Ca²⁺ fluctuation, inositol lipid metabolism, protein kinase C, MAP kinase and phosphatidyl inositol 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.

Note: This product was originally produced by MBL International.

Protocol: Flow Cytometric Analysis for Adherent Cells

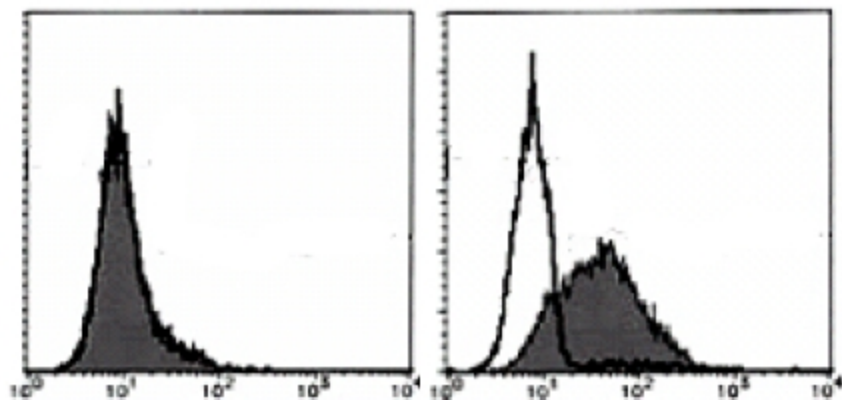
We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).

- 1) Detach the cells from the culture dish by using cell dissociation buffer
- 2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃].
- 3) Resuspend the cells with washing buffer (5x10⁶ cells/ml).
- 4) Add 50 µl of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at RT (20~25°C). Remove supernatant by careful aspiration.
- 5) Add 10 µl of normal goat serum containing 1 mg/ml normal human IgG and 0.1% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 6) Add 40 µl of the primary antibody at the concentration suggested in the APPLICATIONS, diluted in the washing buffer. Mix well and incubate for 30 minutes at RT.
- 7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at RT. Remove supernatant by careful aspiration.
- 8) Add 30 µl of 1/100 FITC conjugated anti-Mouse IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at RT
- 9) Add 1 ml of the washing buffer followed by centrifugation at 500 x g for 1 minute at RT. 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 suggest in the APPLICATIONS onto the cells and incubate for 1 hour at RT. (Optimization of antibody concentration or incubation condition are 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 plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat wash once more.
- 3) Add 30 µl of 1/40 FITC conjugated anti-mouse IgG diluted with PBS onto the cells. Incubate for 30 minutes at RT. Keep out light by covering with aluminum foil.
- 4) Wash the slide in plenty of PBS as in 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 Permafluor™ aqueous mounting medium onto the slide, then put a cover slip on it.

Product images:

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 ITGA7 antibody to the cells.