Product No. F-3152
Lot 105H6711

Monoclonal Anti-Mouse CD11a (LFA-1a) FITC Conjugate

Purified Rat Immunoglobulin
Clone I21/7

Monoclonal Anti-Mouse CD11a (LFA-1a) (rat IgG2a isotype) is derived from the I21/7 hybridoma produced by the fusion of MPC.11.TG.1.7.Oua myeloma cells with splenocytes from a Lewis rat immunized with BW 5147 cell line. The product is provided as purified immunoglobulin conjugated to fluorescein isothiocyanate isomer I. The conjugate is purified by gel filtration to remove unconjugated FITC and antibody. No free FITC or antibody is detectable. The product is provided at a concentration of 0.25 mg/ml in 0.01 M phosphate buffered saline, pH 7.2, containing 1% BSA, 2 mM EDTA and 0.1% sodium azide (see MSDS)* as a preservative.

Description

FITC Conjugated Monoclonal Anti-Mouse CD11a (clone I21/7) is specific for CD11a, the alpha chain of the CD11a-CD18 complex. CD11a is a glycoprotein with a molecular weight of 26 kD expressed in combination with the CD18 beta chain. The complex is a member of the beta2 integrin family. These molecules function in cell adhesion and specifically bind to CD54, ICAM-2, ICAM-3. Anti-CD11a from I21/7 reacts with 97% of thymocytes, 95% of blood lymphocytes, 70% of bone marrow cells of all mouse strains tested.

Performance

When assayed by flow cytometry, approximately 1 µg (4 µl) of the product will stain 1 x 10^6 mouse spleen cells to maximum fluorescence intensity and percent positive. Prior to adding the product to cells, it is recommended that cell surface Fc receptors be blocked by incubating the cells with 10% - 20% normal goat serum (Sigma Product No. G-9023) for 10 minutes at 4°C. A stabilizing protein such as 1% BSA should be included in the diluent when making dilutions of this product.

F/P Molar Ratio: 3.6

Uses

FITC Conjugated Monoclonal Anti-Mouse CD11a may be used for:

1. Direct immunofluorescence analysis of mouse cells by flow cytometry or microscopy.
2. Simultaneous multicolor analysis when used in conjunction with PE or Quantum Red™ conjugates.

Storage

Store at 2-8°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

* Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Procedure for Direct Immunofluorescent Staining of Splenocytes or Thymocytes

Reagents and Materials Needed but not Supplied

1. Diluent: 0.01 M Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
2. FITC conjugated, isotype-matched, non-specific rat or mouse immunoglobulin (Sigma Product No. F-6522).

Procedure

Notes:

1. In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum dilution of antibody by titration assay.

2. Flow cytometric analysis of rodent cells yield better results when the cells are kept cold. Therefore, pre-chill all buffers and diluents, and keep the cells on ice during preparation and staining steps.

1. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., Trypan Blue, Sigma Product No. T-0776). For each sample, add 100 µl or 1 x 10^6 cells per tube. (Note: If cell preparation contains red blood cells, they can be lysed by incubating the cells in approximately 10 mls of 0.017 M Tris, 0.75% NH_4Cl, pH 7.2 at room temperature for 5 - 10 minutes followed by centrifugation and washing 2 times in diluent).
2. Add 1 µg (4 µl) of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at 4°C for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: diluent in place of monoclonal antibody, followed by steps 3 - 8.
   b. Negative staining control: FITC conjugated, isotype-matched, non-specific rat or mouse immunoglobulin at the same concentration as test antibody, followed by steps 3 - 8.
3. After 30 minutes, add 2 ml of cold diluent to all tubes.
4. Pellet cells by centrifugation at 500 x G, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml of cold diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. Resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde if cells are to be stored before analyzing.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and/or secondary antibodies. The best negative control reagent is a FITC conjugated, isotype-matched, rat or mouse monoclonal antibody or myeloma protein. It should not be reactive with the cells being analyzed and should be used at the same concentration as the fluorophore conjugated specific antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

References

Trowbridge, I., and Omary, B., J. Exp. Med., **154**, 1517 (1981).