SIGMA CHEMICAL COMPANY

Product No. F-7275
Lot 017H0157

Monoclonal Anti-Mouse CD3 FITC Conjugate

Purified Rat Immunoglobulin
Clone 29B

Monoclonal Anti-Mouse CD3 (rat IgG2b isotype) is derived from the 29B hybridoma produced by the fusion of the X63Ag8.653 murine cell line with splenocytes from a Lewis rat immunized with a mouse Th2 cell line. The product is provided as protein A purified antibody 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, 2 mM EDTA, pH 7.2, containing 1% BSAand 0.1% sodium azide (see MSDS)* as a preservative.

Description

FITC Conjugated Monoclonal Anti-Mouse CD3 recognizes the 25 kD epsilon chain of the CD3 complex of integral membrane proteins. The CD3 complex consists of at least five polypeptide chains (gamma, delta, epsilon, neu and zeta) and is physically associated with the alpha/beta or gamma/delta T cell antigen receptor. The CD3 complex is involved in T cell activation by mediation or amplification of signals triggered by the alpha/beta or gamma/delta T cell receptor. CD3 is a pan T cell marker and recognizes 100% of T cells in 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 Ratio: 4.7

Uses

FITC Conjugated Monoclonal Anti-Mouse CD3 may be used for:

1. Direct immunofluorescence analysis of mouse T cells by flow cytometry or fluorescence 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-6647).

Procedure

Notes:
1. In order to obtain best results in different preparations, it is recommended that each individual user determine the optimum amount of antibody required for their preparation 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 1×10^7 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 1×10^6 cells per tube. (Note: If the 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 on ice 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 by 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

Portoles, P., et al., J. Immunol, **142**, 4169 (1989).
Holmes, K., and Morse III, H., Immunology Today, **9**, 344 (1988).
Unkless, J., J. Exp. Med., **150**, 580 (1979).

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