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ProductInformation

MONOCLONAL ANTI-HUMAN CD8
FITC CONJUGATE
Clone UCHT-4
Purified Mouse Immunoglobulin

Product Number **F 0772**

Product Description

Monoclonal Anti-Human CD8 (mouse IgG2a isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from Balb/c mice immunized with human thymocytes followed by peripheral blood T cells. The isotype is determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with purified CD8 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC. No free FITC is detectable.

Monoclonal Anti-Human CD8 recognizes the CD8 30/32 kD human T cytotoxic/suppressor lymphocytes surface glycoprotein. The CD8 antigen is strongly expressed on approximately one-third of mature T cells (cytotoxic/suppressor T cells). In suspension about 90% of thymocytes will be stained while cortical and medullar sections of thymus will also show staining. A subset of NK cells express this antigen somewhat weakly. Monoclonal Anti-CD8 does not stain B lymphocytes, monocytes, or granulocytes. The epitope recognized by this clone is sensitive to routine formalin fixation and paraffin embedding. Cryostat sections post fixed in formalin can also be stained.

FITC Monoclonal Anti-Human CD8 may be used for:
1. Enumeration of total T cytotoxic/suppressor lymphocytes in bone marrow, blood and other body fluids.
2. Identification and localization of T cytotoxic/ suppressor lymphocytes in lymphoid and other tissues.
3. Analysis of cell mediated cytotoxicity.
4. Studies of immunoregulation in health and disease.
5. Investigation of NK cells.
6. Complement mediated cytolysis of CD8 expressing cells.

Reagents

The antibody is provided as a solution (25-200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative.

Precautions and Disclaimer

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 hazards and safe handling practices.

Storage/Stability

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.

Procedure

Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied
1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A, or heparin anticoagulant **OR**
   b. Human cell suspension (peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA, and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F 6522).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T 0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **OR**
   b. Adjust cell suspension to 1 x 10^7 cells/ml in Diluent. Cells should be >90% viable as determined by dye exclusion (trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 to 22 °C) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 – 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. F 6522) at the same concentration as test antibody followed by steps 3 – 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of Diluent to all tubes.
5. Pellet cells by centrifugation at 500 x *g* for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the 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.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x 10^6, cells a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

F/P Molar Ratio: range of 3-8

Note: In order to obtain best results in different preparations it is recommended that each individual user determine their optimum working dilutions by titration assay.

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