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ProductInformation

MONOCLONAL ANTI-HUMAN CD3
FITC CONJUGATE
Clone UCHT-1
Purified Mouse Immunoglobulin

Product Number **F 0522**

Product Description

Monoclonal Anti-Human CD3 (mouse IgG1 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 Sezary T cells. The isotype is determined using 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 to purified CD3 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

FITC Conjugated Monoclonal Anti-Human CD3 recognizes the CD3 complex which is composed of 5 chains designated γ, δ, ε, ζ and η having a molecular mass distribution of 16, 20, and 25-28 kDa. The CD3 human lymphocyte surface antigen is a glycoprotein thought to be associated with the T cell antigen receptor and to be involved in transmission of activation signals. The CD3 antigen is present on 60-80% of normal peripheral blood mononuclear cells, 20-40% of normal spleen cells, 40% of normal thymocytes, the majority of T-CLL and approximately 70% of T-ALL. The antibody stains the cytoplasm of cerebellar Purkinje cells but does not stain B lymphocytes, monocytes, granulocytes, or NK cells. The epitope recognized by clone UCHT-1 is expressed on the ε-chain of the CD3 antigen/T cell receptor complex. Detection of the epitope appears to be dependent of the bindign to CD3-γ or CD3-δ. The epitope is sensitive to routine formalin fixation and paraffin embedding. Cryostat sections post-fixed in formalin can also be stained.

Reagents

The conjugate is provided as a solution 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 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 (e.g., peripheral blood mononuclear cells isolated on Histopaque® (Product Code 1077-1)).
2. Diluent: 0.01M 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 6397).
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 phosphate buffered saline, 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 to mix. 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 of Diluent in place of monoclonal antibody followed by steps 3 - 8.
b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.

3. After 30 minutes add 2 ml of 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 Diluent.
7. Repeat washing procedure (steps 3-6) twice. Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
8. After last wash, resuspend cells in 0.5 ml of Diluent or 2% paraformaldehyde (if cells are stored before analyzing) and 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 staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary 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.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems, it may be necessary to incubate the cells (at step 2 before adding monoclonal antibody) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

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.

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

1. Enumeration of total T lymphocytes in bone marrow, blood and other body fluids.
2. Identification and localization of normal and malignant T lymphocytes, lymphoid and other tissues.
3. Identification of leukemias and lymphomas of T cell origin.
4. T lymphocyte activation studies.

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.

References

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