

Product Information

Anti-CD4-FITC antibody, Mouse monoclonal
clone Q4120, purified from hybridoma cell culture

Product Number F1773

Product Description

Anti-CD4-FITC antibody, Mouse monoclonal (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 CD4-Transfected mouse T cell hybridoma, 3DT, followed by CD4⁺ human T cell CEM cells. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD4 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

FITC Conjugated Monoclonal Anti-Human CD4 may be used for:

1. Identification, quantification and monitoring of helper/inducer T cells in peripheral blood, biological fluids, lymphoid organs, and other tissues.
2. Analysis of T cell mediated cytotoxicity.
3. Characterization of subtypes of T cell leukemias and lymphomas.
4. Studies of T cells in health and disease.

Anti-CD4-FITC antibody, Mouse monoclonal recognizes the CD4 human cell surface glycoprotein (59 kDa) which belongs to the immunoglobulin superfamily. It is expressed on the helper/inducer T cell subset, which is found on the majority of peripheral blood lymphocytes (PBLs), most cortical and mature medullary thymocytes, microglial cells, dendritic cells, and on some malignancies of T cell origin. Lower levels of CD4 have been detected in monocytes, macrophages and granulocytes. The CD4 molecule binds to the major histocompatibility complex (MHC) class II molecules during the interaction of CD4⁺ T cells with antigen presenting cells or with target cells. It also serves as a high affinity cellular receptor for the GP 120 envelope glycoprotein of the human immunodeficiency virus (HIV-1, HIV-2). The cytoplasmic portion of the CD4 molecule is associated with the src related T cell specific P56^{lck} protein kinase. The CD4 molecule is involved in the adhesion of T lymphocytes to target cells, thymic development, transmission of intracellular signals during

T cell activation and binding to polyclonal immunoglobulins. Immunoregulatory T cell subset abnormalities in autoimmunity disorders, immunodeficiency diseases, graft versus host disease and following immunosuppressive therapy are often manifested as a change in CD4⁺/CD8⁺ ratio in peripheral blood T cells. Anti-CD4-FITC antibody, Mouse monoclonal blocks the HIV receptor and prevents syncytium formation. The epitope recognized by the Q4120 clone is located in 1 + 2 domains, i.e., amino acid residues 1-183 and is sensitive to formalin fixation and paraffin embedding. The Anti-CD4-FITC antibody, Mouse monoclonal has been shown to be very similar to anti-Leu3a, clone SK3.

Reagents

The conjugate is provided (100 µg/mL) 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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product Profile

F/P Molar Ratio: 3 to 8

When assayed by flow cytometric analysis, using 10 µL of the antibody to stain 1 x 10⁶ 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.

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.

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.

Procedure

Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A, or heparin anticoagulant **OR**
 - Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code 1077-1)).
- Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
- FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F6397).
- 12 x 75 mm test tubes.
- Adjustable micropipette.
- Centrifuge.
- Counting chamber.
- Trypan blue (Product No. T0776), 0.2% in 0.01 M PBS, pH 7.4.
- 2% paraformaldehyde in PBS.
- Whole blood lysing solution.
- Flow cytometer.

Procedure

- Use 100 µL of whole blood **OR**
 - Adjust cell suspension to 1×10^7 cells/mL in diluent. Cells should be >90% viable as determined by dye exclusion (trypan blue). For each sample, add 100 µL or 1×10^6 cells per tube.
- 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:
 - An autofluorescence control: 10 µL diluent in place of monoclonal antibody, followed by steps 3 - 7.
 - A negative staining control: 10 µL of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. F6397) at the same concentration as test antibody followed by steps 3 - 7.

- If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
 - If a mononuclear cell suspension is used, proceed to Step. 4.
- Add 2 mL of diluent to all tubes.
- Pellet cells by centrifugation at 500 x g for 10 minutes.
- Remove supernatant by careful aspiration.
- 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.

References

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