

Product Information

Monoclonal Anti-CD4-PE, clone Q4120
produced in mouse, purified immunoglobulin

Catalog Number **P7562**

Product Description

Monoclonal Anti-CD4 (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 line CEM cells. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO-2. The product is prepared by conjugation of R-Phycoerythrin (R-PE) with purified CD4 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound R-PE and antibody. No free R-PE or free antibody is detectable.

Monoclonal Anti-CD4 recognizes the CD4 human cell surface glycoprotein (59 kDa) which belongs to the immunoglobulin superfamily. It is expressed on the helper/inducer T subset which comprises the majority of peripheral blood lymphocytes (PBLs), on most cortical and mature medullary thymocytes, on microglial cells, on 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 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 adhesion of T lymphocytes to target cells,, thymic development, transmission of intracellular signals during T cell activation and in binding to polyclonal immunoglobulins. Immunoregulatory T cell subset abnormalities in autoimmunity disorders, in immunodeficiency diseases, in graft versus host disease, and following immunosuppressive therapy are often manifested as a change in CD4⁺/CD8⁺ ratio in peripheral blood T cells. Monoclonal Anti-Human CD4 antibody 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 Monoclonal Anti-Human CD4 antibody

has been shown to be very similar to anti-Leu3a, clone SK3.

Reagents

Supplied as a solution (10 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% 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 Material Safety Data Sheet 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®; Catalog Number 1077-1.
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
3. R-PE conjugated, isotype-matched, non-specific mouse immunoglobulin - negative control, Catalog Number P4685.
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog Number 302643, 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 1×10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 μ l or 1×10^6 cells per tube.
2. Add 10 μ l of conjugate to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes.
Proper controls to be included for each sample are:
 - a. An autofluorescence control: 10 μ l diluent in place of monoclonal antibody followed by steps 3 - 8.
 - b. A negative staining control: 10 μ l of R-PE 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 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

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.
5. Isolation, enrichment, or depletion of helper/inducer T lymphocytes.

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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