MONOCLONAL ANTI-HUMAN CD4
Clone Q4120
Purified Mouse Immunoglobulin

Product Number C 1805

Product Description
Monoclonal Anti-Human 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 CEM 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).

Monoclonal Anti-Human CD4 recognizes the CD4 human cell surface glycoprotein (59 kD) 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 P56lk 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 autoimmune 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. 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.

1. Monoclonal Anti-Human CD4 may be used for:
   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.

Reagents
The product is provided as a solution (50-200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 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
For continuous use, 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
Indirect 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: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%NaN₃.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g., FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab’)2 fragment of Affinity Isolated Antibody, Product No. F 2883). Aggregates in conjugates should be removed by centrifugation immediately prior to use.

4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. M 5284).

5. 12 x 75 mm test tubes.

6. Adjustable micropipet.

7. Centrifuge.

8. Counting chamber.

9. 0.2% Trypan blue (Sigma Product No. T 0776) in 0.01 M phosphate buffered saline, pH 7.4.

10. 2% paraformaldehyde in PBS.

11. Whole blood lysing solution.

12. 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 (e.g., trypan blue).

2. Add 5 µl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 - 22 °C) for 30 minutes.

   Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
   b. Negative staining control 1: 5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M 5284) at the same concentration as test antibody.

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 4-6).

8. After the second wash, resuspend the cells in 100 µl of the fluorochrome-conjugated secondary antibody at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.

9. a. If whole blood is used, use lysing solution after incubation according to manufacturer’s instructions, then proceed to Step 10.
   b. If a mononuclear cell suspension is used, proceed to Step 10.

10. Add 2 ml diluent to all tubes.

11. Wash as in steps 4-6 twice.

12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer’s instructions.

Quality Control

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 in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

Product Profile

When assayed by flow cytometric analysis, using 5 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.

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