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ProductInformation

MONOCLONAL ANTI-HUMAN CD4 BIOTIN CONJUGATE
CLONE Q4120
Purified Mouse Immunoglobulin

Product No. **B7280**

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 T cell hybridoma followed by $CD4^+$ human T cell line CEM.[1,2] The isotype is determined using the Sigma Immuno-Type™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of ε-amino-caproyl-biotin to purified CD4 monoclonal antibody. The conjugate is provided as purified immunoglobulin (50-200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15mM sodium azide (see MSDS)* as a preservative.

Description

Monoclonal Anti-Human CD4 antibody recognizes the CD4 59 kD human cell surface glycoprotein. CD4 is a single chain transmembrane glycoprotein which belongs to the immunoglobulin superfamily.[3,4,5,6,7,8] It is expressed on the helper/inducer T subset which comprises the majority of peripheral blood lymphocytes on most cortical and mature medullary thymocytes, microglial cells, dendritic cells and 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 part of the CD4 molecule is associated with the src related T cell specific $P56^{lck}$ protein tyrosine 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, 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 monoclonal Q4120 is located in the 1 + 2 domains (i.e., amino acid residues 1-183) and is sensitive to formalin fixation and paraffin embedding. Monoclonal Anti-Human CD4 antibody is very similar to anti-Leu3a (Clone SK3).[1,2]

Performance

When assayed by flow cytometric analysis, using 10 µl of the antibody per 1 X 10^6 cells and ExtrAvidin®-FITC Conjugate (Product No. E-2761), 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.

Uses

Biotin Monoclonal Anti-Human CD4 antibody 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 T cell leukemias and lymphomas.
4. Studies of T cells in health and disease (e.g., Immunodeficiency states).

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

Storage/Stability
Store at 2-8 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

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 hazardous and safe handling practices.

Procedure for Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

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® Sigma Stock No. 1077-1).
2. Diluent: 0.01 M Phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin®-FITC (Sigma Product No. E2762), Streptavidin-FITC (Sigma Product No. S3402), Streptavidin-PE (Sigma Product No. S3762), or Streptavidin-Quantum Red™ (Sigma Product No. S2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Sigma Product No. T0776) 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 (e.g., trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of biotinylated monoclonal antibody 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.
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) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated avidin derivative 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. Centrifuge and wash as in steps 4 - 6 twice.
11. 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.

References

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