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Product Information

Monoclonal Anti-CD4- Quantum Red™
Clone Q4120
produced in mouse, purified immunoglobulin

Catalog Number **R8886**

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 CEM cells. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of Quantum Red with purified CD4 monoclonal antibody. Quantum Red is Sigma's tandem fluorochrome in which a small organic dye, Cy5, is covalently linked to R-Phycoerythrin (PE). The PE absorbs light energy at 488nm and emits in the excitation range of Cy5 which acts as the acceptor dye. The complex then emits at 670 nm.

Monoclonal Anti-CD4-Quantum Red 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.

Monoclonal Anti-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 $P56^{lck}$ 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 immuno-suppressive therapy are often manifested as a change in $CD4^+/CD8^+$ ratio in peripheral blood T cells. Monoclonal Anti-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-CD4 antibody has been shown to be very simlar to anti-Leu3a, clone SK3.

Reagents
Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide as a preservative.

Precautions
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.

Performance
Assayed by flow cytometric analysis using 10 µl of the conjugate to stain 1×10^6 cells. Maximum signal to background and maximum percent positive are obtained.

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.

A_{567}/A_{280}: 1 - 4

Storage
Store at 2-8 ºC. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure for 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 10771.
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. QR conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog Number R2138).
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 PBS, 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 conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 - 22ºC) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of QR conjugated, isotype-matched non-specific mouse immunoglobulin, Catalog Number R2138, at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. 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 binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein that 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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