3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ANTI-HUMAN CD3-FITC/CD4-PE
DUAL-TAG™
Clones: UCHT-1/Q4120

Product Number **F 7152**

Product Description

Monoclonal Anti-Human CD3 (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 human thymocytes followed by Sezary T cells. The product is prepared by the conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD3 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC.

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, 3DT, followed by $CD4^+$ human T cell line, CEM. The product is prepared by conjugation of R-Phycoerythrin (PE) with purified CD4 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE and antibody, no free antibody is detectable.

Monoclonal Anti-Human CD3 recognizes the CD3 complex which is composed of 5 chains designated γ, δ, ε, ζ, and η having a molecular mass distribution of 16, 20 and 25 - 28 kDa. The CD3 human lymphocyte surface antigen is a glycoprotein thought to be associated with the T cell antigen receptor and to be involved in transmission of activation signals. The CD3 antigen is present on 60-80% of normal peripheral blood mononuclear cells, 20-40% of normal spleen cells, 40% of normal thymocytes, the majority of T-CLL and approximately 70% of T-ALL. The antibody stains the cytoplasm of cerebellar Purkinje cells but does not stain B lymphocytes, monocytes, granulocytes, or NK cells. The epitope recognized by clone UCHT-1 is expressed on the ε-chain of the CD3 antigen/T cell receptor complex. Detection of the epitope appears to be dependent of the binding to CD3-γ or CD3-δ. The epitope is sensitive to routine formalin fixation and paraffin embedding. Cryostat sections post-fixed in formalin can also be stained. Monoclonal Anti-Human 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 on 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 dieseases, 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 the 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-Leu-3a, clone SK3.

Anti-Human CD3-FITC/CD4-PE DUAL TAG may be used for:

1. Identification and enumeration of helper/inducer T cell subsets.
2. Classification of subtypes of T cell leukemias and lymphomas.
3. Studies of T cells in health, HIV infection and AIDS, and other associated diseases.

Reagents

The two conjugates are provided as a pre-titered 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
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 hazardous 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® (Product Code 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
4. FITC and PE conjugated, isotype-matched, non-specific mouse immunoglobulins.
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T 0776), 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 20 µl of conjugates 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. An autofluorescence control: 20 µl diluent in place of monoclonal antibodies, followed by steps 3 - 7.
   b. A negative staining control: 20 µl of FITC and PE conjugated, isotype-matched, non-specific mouse immunoglobulins at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation 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. Proper color compensation is important for unbiased data interpretation. Cell samples stained with the corresponding single reagents of the pair may be used as controls for adjusting compensation. Alternatively, microbead standards may be used (Flow Cytometry Compensation Kit, Product code COMP-1).

Quality Control
It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the antibodies. The ideal negative control reagent is a combination of a FITC- and PE-conjugated mouse monoclonal or myeloma proteins which have no reactivity with human cells. It should be isotype-matched to the antibodies in the DUAL TAG antibody reagent and of the same concentration and F/P molar ratio as the DUAL TAG antibody reagent. 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.

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