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Product Information

Monoclonal Anti-CD3, clone UCHT-1
produced in mouse, purified immunoglobulin

Catalog Number **C7048**

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 an immunized mouse. Human thymocytes followed by Sezary T cells were used as the immunogen. The isotype is determined using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

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. It is detectable in the cytoplasm of cortical thymocytes but also appears on the surface of medullary thymocytes. 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 be stained.

Reagent

Supplied as purified antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15mM 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. 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® (Catalog Number 10771)).
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 anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Catalog No. F2883, Anti-Mouse IgG (whole molecule), $F(ab')_2$ fragment-FITC). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog Number M5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipette.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Catalog No. 302643) 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 (trypan blue). For each sample, add 100 µL or 1 x 10^6 cells per tube.

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 to 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: 5 µL isotype-matched non-specific mouse immunoglobulin (Catalog No. M5284) 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 of 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 of 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

Monoclonal Anti-Human CD3 may be used for:

1. Enumeration of total T lymphocytes in bone marrow, blood and other body fluids.
2. Identification and localization of normal and malignant T lymphocytes and lymphoid and other tissues.
3. Identification of leukemias and lymphomas of T cell origin.
4. T lymphocyte activation studies.
5. Immunoprecipitation of the CD3 antigen.

When assayed by flow cytometric analysis, 5 µL of the monoclonal antibody will stain 1 x 10^6 cells with a fluorescence intensity and percent positive that is similar to that observed with saturating amounts of monoclonal antibody.

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.

References

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