Product No. C-7423
Lot 096H4858

Monoclonal Anti-Human CD8
Purified Mouse Immunoglobulin
Clone UCHT-4

Monoclonal Anti-Human CD8 (mouse IgG2a 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 peripheral blood T cells. The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

Monoclonal Anti-Human CD8 recognizes the CD8 30/32 kD human T cytotoxic/suppressor lymphocyte surface glycoprotein. The CD8 antigen is strongly expressed on approximately one-third of mature T cells (cytotoxic/suppressor T cells). In suspension, about 90% of thymocytes will be stained while cortical and medullar sections of thymus will also show staining. A subset of NK cells express this antigen somewhat weakly. Monoclonal Anti-CD8 does not stain B lymphocytes, monocytes or granulocytes. The epitope recognized by this clone is sensitive to routine formalin fixation and paraffin embedding.

Performance

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.

Uses

Monoclonal Anti-Human CD8 may be used for:
1. Enumeration of total T cytotoxic/suppressor lymphocytes in bone marrow, blood and other body fluids.
2. Identification and localization of T cytotoxic/suppressor lymphocytes in lymphoid and other tissues.
3. Analysis of cell mediated cytotoxicity.
4. Studies of immunoregulation in health and disease.
5. Investigation of NK cells.
6. Complement mediated cytolysis of CD8 expressing cells.

Storage

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

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.

*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.

Procedure for 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® (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 anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Sigma Product No. F-2883 FITC-Sheep Anti-Mouse IgG (whole molecule), $F(ab')_2$ fragment of Affinity Isolated Antibody). 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-5409).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.

Reagents and Materials Needed but Not Supplied (cont.)

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). 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 - 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 (Sigma Product No. M-5409) 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.

Selected References

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