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Product Information

Monoclonal Anti-CD8–Quantum Red™
Clone UCHT-4
produced in mouse, purified immunoglobulin

Catalog Number **R6260**

Product Description

Monoclonal Anti-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 the Sigma Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of Quantum Red with purified CD8 monoclonal antibody. Quantum Red is Sigma's tandem fluorochrome in which a small organic dye, Cy5, is covalently linked to R-Phycoerythrin (R-PE). The R-PE absorbs light energy at 488 nm and emits in the excitation range of Cy5 which acts as the acceptor dye. The complex then emits at 670nm. The principle of energy transfer fluorochromes was first described by Glazer, et al. The conjugate is purified by gel filtration to remove unbound Quantum Red and antibody. No free Quantum Red or free antibody is detectable.

Uses

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, e.g., investigation of NK cells.

Monoclonal Anti-CD8 recognizes the CD8 30/32 kDa human T cytotoxic/suppressor lymphocytes surface glycoprotein. The CD8 antigen is strongly expressed on approximately one-third of mature T cells (cytotoxic/suppressor T cells). In suspension, ~90% of thymocytes will be stained in addition to cortical and medullar sections of thymus. 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. Cryostat sections post fixed in formalin can also be stained.

Reagent

The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative.

Precautions and 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.

Storage

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

Note: Store product protected from light.

Product Profile

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

F/P Molar Ratio: 0.5 to 3.0

Note: In order to obtain the best results in various preparations, it is recommended that each individual user determine their optimum working dilutions by titration.

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®, Catalog Number 10771.

2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA, and 0.1% NaN_3.
3. Quantum Red conjugated, isotype-matched, non-specific mouse immunoglobulin.
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog Number T0776, 0.2% 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 (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 to mix. Incubate the cells at room temperature 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 3-8.
   b. A negative staining control: 10 µL of Quantum Red conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3-8.
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 3-6) twice. **Note**: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
8. 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.

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 (at step 2 before adding monoclonal antibody) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

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