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ProductInformation

MONOCLONAL ANTI-HUMAN CD8
BIOTIN CONJUGATE CLONE UCHT-4
Purified Mouse Immunoglobulin

Product Number **B 0406**

Product Description

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 the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of ε-amino caproyl biotin with purified CD8 monoclonal antibody.

Monoclonal Anti-Human 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, 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. Mono-clonal 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.

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x 10^6 cells, a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

Reagents

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 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 hazards 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

Procedure for Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

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.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin®-FITC (Product No. E 2762), Streptavidin-FITC (Product No. S 3402), Streptavidin-PE (Product No. S 3762), or Streptavidin-Quantum Red™ (Product No. S 2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Product No. T 0776) 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 (e.g., trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of biotinylated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes.

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) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated avidin derivative 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. Centrifuge and wash as in steps 4 - 6 twice.
11. 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.

Product Profile

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

Biotin 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.

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