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ProductInformation

Anti-Human CD3-FITC/CD8-PE
DUAL-TAG™
Clones: UCHT-1/UCHT-4

Product No. **F 7027**

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, no free FITC is detectable.
F/P Molar Ratio: 3 to 5
Concentration: Minimum 20 µg/ml

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 product is prepared by conjugation of R-Phycoerythrin (PE) with purified CD8 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE and antibody, no free PE or free antibody is detectable.
A_{567}/A_{280}: 1 to 3
Concentration: Minimum 20 µg/ml

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 kD. 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 bindign 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 CD8 recognizes the CD8 30/32 kDa 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 and 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. Cryostat sections post fixed in formalin can also be stained.

Anti-Human CD3-FITC/CD8-PE DUAL TAG may be used for:
1. Identification and enumeration of $CD8^+/CD3^+$ cytotoxic/suppressor T lymphocytes with discrimination of $CD8^+CD3^-$ Nk cells.
2. Enumeration of "Helper/Suppressor" T Lymphocyte ratio when used in parallel with Anti-Human CD3-FITC/CD4-PE DUAL TAG (Sigma Product No. F 7152).
3. Classification of subtypes of T cell leukemias and lymphomas.
4. 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, pH7.4, containing 1% BSA with 0.1% sodium azide as a preservative.

Precautions

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.

Product Profile

When assayed by flow cytometric analysis using 20 µl of the antibody to stain 1 x 10^6 cells, a fluorescence intensity for each antibody conjugate is observed similar to that obtained with saturating monoclonal antibody levels of each conjugate in single color flow cytometry.

Storage
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 for 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? (Sigma Product No. 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC and PE conjugated, isotype-matched, non-specific mouse immunoglobulins (negative control, FITC Mouse IgG1/PE Mouse IgG2a, Product No. F 0528).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T0776), 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 - 22EC) 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 (Product No. F0528), 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, micro-bead standards may be used (Flow Cytometry Compensation Kit, Sigma Stock No. 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 auto-fluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

Selected References

Barber, E., et al., Proc. Natl. Acad. Sci. (USA), **86**, 3277 (1989).
Beverley, P., et al., Eur. J. Immunol., **11**, 329 (1981).
Biddison, W., et al., Human Immunol., **9**, 117 (1984).
Borst, J., et al., J. Biol. Chem., **258**, 5135 (1983).
Burns, G., et al., J. Immunol., **129**, 1451 (1982)
Callard, R., et al., Clin. Exp. Immunol., **43**, 49 (1981).
Fischer, A., et al., Immunol., **48**, 177 (1983).
Furley, A., et al., Cell, **46**, 75 (1986).
Garson, J., et al., Nature, **298**, 375 (1982).
Glazer, N., et al., J. Biophys. Soc., **43**, 383 (1982).
Gold, D., et al., Nature, **321**, 4 (1986).
Kavanthas, P., et al., Proc. Natl. Acad. Sci. (USA), **81**, 7688 (1984).
Khin, Y., et al., Int. J. Cancer, **36**, 433 (9185).
Knapp, W., et al. (eds.), Leukocyte Typing IV, Oxford Press, New York, 1075 (1989).
Littman, S., et al., Cell, **40**, 237 (1985).
Martin, P., et al., J. Immunol., **132**, 759 (1984).
Merkenschlager, M., et al., Eur. J. Immunol., **18**, 1653 (1988).
Merkenschlager, M., Int. Immunol., **1**, 450 (1989).
Meuer, S., et al., Nature, **303**, 808 (1983).
Oettgen, H., et al., Ann. Rev. Immunol., **6**, 629 (1988).
Pozilly, P., et al., Diabetes, **32**, 91 (1983).
Reinherz, E., et al., J. Immunol., **124**, 1301 (1980).
Reinherz, E., et al., Nature, **294**, 168 (1981).
Reinherz, E., et al., Cell, **30**, 715 (1982).
Rowe, D., et al., Clin. Exp. Immunol., **54**, 327 (1983).
van dan Elsen, P., et al., Nature, **312**, (1984).

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