Monoclonal Anti-human CD3ε-Phycoerythrin

Catalog Number: FAB100P
100 Tests

Reagent Information

Phycoerythrin-conjugated mouse monoclonal anti-human CD3ε: Contains 1.0 mL of PE-labeled antibody at a concentration of 25 μg/mL.

Clone #: UCHT1

Ig Class: mouse IgG_1

Storage: 2 - 8° C

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD3 within a population and qualitatively determine the density of CD3 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, that binds to cells expressing CD3. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing CD3 are fluorescently stained, with the intensity of staining directly proportional to the density of CD3. Cell surface expression of CD3 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human CD3: Use as is; no preparation is necessary.

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Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 μL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should be Fc-blocked by treatment with 1 μg of mouse or human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-CD3 reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CD3 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems' Human Erythrocyte Lysing Kit, Cat. # WL1000*).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with PE-labeled murine IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

The structure and function of the T cell receptor (TCR)/CD3 complex have been extensively reviewed (1 - 3). The TCR/CD3 complex consists of two variable antigen recognition receptor chains, either TCR-α/TCR-β or TCR-γ/TCR-δ, that are non-covalently linked to at least four different invariant chains, CD3γ, CD3δ, CD3ε, and the CD3ζ chain that exists as a homodimer or as a heterodimer with its splice-variant the CD3η chain. The CD3 genes are expressed early during thymocyte development, before the rearrangement and expression of the TCR genes (4, 5).

The human CD3ε gene was isolated and characterized from a genomic library of peripheral blood mononuclear cells screened with a cDNA clone (6). The CD3γ, CD3δ and CD3ε genes are clustered on q23 of chromosome 11 and are hypothesized to have arisen via a gene duplication event (7). The UCHT1 monoclonal anti-T cell antibody was produced from a mouse hybridoma following immunization with human infant thymocytes (8). This antibody has been found to react with the CD3ε chain of the TCR/CD3 complex (9). Furthermore, immobilized UCHT1 monoclonal antibody has been shown to induce T cell proliferation (9), illustrating the role for CD3 in signal transduction during antigen recognition.

References

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2. Davis, M.M. (1990) Ann. Rev. Biochem. **59**:475.
3. Cambier, J.C. (1992) Curr. Opin. Immunol. **4**:257.
4. Furley, A.J. *et al*. (1986) Cell **46**:75.
5. van Dongen, J.J. *et al*. (1987) J. Immunol. **138**:1260.
6. Clevers, H.C. *et al*. (1988) Proc. Natl. Acad. Sci. USA **85**:8156.
7. van den Elsen, P. *et al*. (1985) Proc. Natl. Acad. Sci. USA **82**:2920.
8. Beverley, P.C. and R.E. Callard (1981) Eur. J. Immunol **11**:329.
9. Schlossman, S. *et al.* (1995) Leukocyte Typing V: White Cell Differentiation Antigens, Oxford University Press, New York.

Note: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.