Monoclonal Anti-mouse CD5-Fluorescein

Catalog Number: FAB115F
Lot Number: LBV02
100 tests

Reagent Information

Carboxyfluorescein-conjugated rat monoclonal anti-mouse CD5: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 53-7.3

Ig class: rat IgG_{2A}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing the CD5 cell surface antigen within a population and qualitatively determine its density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to the cells expressing CD5. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing CD5 are fluorescently stained, with the intensity of staining directly proportional to the density of CD5. Cell surface expression of CD5 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated rat anti-mouse CD5: Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell supension. 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells 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) are 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse IgG/10^5 cells for 15 minutes at room temperature. 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 fluorescein-conjugated anti-mouse CD5 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD5 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
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 fluorescein-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

Mouse CD5, formerly known as Ly-1 or Lyt-1 (2), is a 67 kDa glycoprotein expressed on thymocytes, T cells and on a small population of B cells (1). Mouse CD5 expression is higher on $CD4^+$ T cells compared to $CD8^+$ T cells (3). Mouse CD5 shares 63% overall amino acid homology with its human counterpart (4). The ligand for CD5 is the C-type lectin, CD72. Both murine and human CD72 are able to interact with mouse CD5 (1). CD5 expression on B cells has been associated with a small population, B-1a, abundant in the peritoneal and plueral cavity (5). These B cells have a predisposition for reacting with self antigens by virtue of the limited antibody repertoire that they are capable of expressing during their lifetime (6). Elevated levels of CD5 expressing B cells have been reported in autoimmune conditions, as well as on some B cell tumors (5, 7). Furthermore, in developing thymocytes, CD5 can act as a negative regulator of T-cell receptor signaling and thereby influence the maturational fate of thymocytes (8). Therefore, the common role of CD5 in both B and T lineages appears to be associated with controlling the hyper reactivity that can occur through B and T cell receptor activation (9).

References

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2. Huang, H.J. *et al*. (1987) Proc. Natl. Acad. Sci. USA **84**:204.
3. Ledbetter, J.A. *et al*. (1980) J. Exp. Med. **152**:280.
4. Jones, N.H. *et al*. (1986) Nature **323**:346.
5. Hayakawa, K. *et al*. (1983) J. Exp. Med. **157**:202.
6. Pennell, C.A. *et al*. (1985) Proc. Natl. Acad. Sci. USA **82**:3799.
7. Lanier, L.L. *et al.* (1981) J. Exp. Med. **153**:998.
8. Tarakhovsky, A. *et al*. (1995) Science **269**:535.
9. Bikah, G. *et al*. (1996) Science **274**:1906.

Warning: 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.