Monoclonal
Anti-mouse CD45 (Ly-5)-Fluorescein

Catalog Number: FAB114F
Lot Number: LBT03
100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated monoclonal anti-mouse CD45: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 30-F11

Ig class: rat IgG_{2B}

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 mouse CD45 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 cells expressing the mouse CD45 antigen. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing the CD45 antigen are fluorescently stained, with the intensity of staining directly proportional to the density of CD45. Cell surface expression of the CD45 antigen is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated rat anti-mouse CD45: Use as is; no preparation is necessary. The investigator should determine the optimal antibody staining concentration by first performing a dilution analysis where decreasing amounts of antibody are used to stain a known tissue sample. Use of excessive amounts of antibody in cell staining reactions can lead to high background signals.

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 CD45 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD45 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) Finally, 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_{2B} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Monoclonal 30-F11 has been shown to react with all isoforms of the CD45 molecule, also known as the Leukocyte Common Antigen, Ly-5, T200 glycoprotein, B220 and receptor-type protein-tyrosine phosphatese C (1). The different isoforms of CD45 were identified as cell lineage, maturational and activation state specific (2). All cells of hematopoietic origin express the CD45 antigen except erythrocytes (3). Therefore, this monoclonal antibody is a useful reagent to distinguish between hematopoietic and non-hematopoietic cells.

References

1. Ledbetter, J.A. and L.A. Herzenberg (1979) Immunol. Rev. **47**:63.
2. Johnson, P.A. *et al*. (1997) Weir's Handbook of Experimental Immunology, Vol. 2; Herzenberg, L.A. *et al*. (editors)
3. Thomas, M.L. (1989) Annu. Rev. Immunol. **7**:339.

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.