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FITC Anti-Mouse Dendritic Cells (Cell Surface) Monoclonal Antibody

CL89145F Lot: 14532

DESCRIPTION:

CL89145F (NLDC-145) identifies Ia positive interdigitating cells, veiled cells, and Langerhans cells of the skin and their in vitro counterparts. The antigen, also known as DEC-205, is expressed at high levels by dendritic cells and thymic epithelial cells. The dendritic cells that express high levels of the antigen are those in the skin, in the T cell regions of peripheral lymphoid organs and dendritic cells that are grown from proliferating bone marrow precursors in the presence of high dose GM-CSF. DEC-205 can also be expressed on B cells, although at much lower levels. It is absent in freshly isolated macrophages from the peritoneal cavity although a sub-population becomes weakly positive in mice previously stimulated with thioglycollate. The antigen detected by CL89145 is an integral membrane glycoprotein with an apparent mass of 205 kDa. DEC-205 is apparently a receptor involved in antigen-processing by dendritic cells. The antigen recognized by CL89145 is localized in the cytoplasm (after fixation) and on the cell surface. It is more widely distributed than the CL89148 (MIDC-8) antigen which is a very specific cytoplasmic component. This clone can be used in flow cytometry, immunohistochemistry, immunocytochemistry, immunoblotting and for functional studies.

PRESENTATION:

100 µg FITC conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles. Avoid prolonged exposure to light.

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SPECIFICITY: Reacts with interdigitating cells, dendritic cells.

CLONE: NLDC-145

ISOTYPE: Rat IgG_{2a}

FORMAT: FITC conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Affinity Purified IgG from culture supernatant)

FLOW CYTOMETRY ANALYSIS:

Method:

- 1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium (CL5030).
- 2. Wash 2 times.
- 3. Resuspend the cells to a concentration of $2x10^7$ cells/ml in media A. Add 50μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
- 4. To each tube, add $1.0 \mu g^*$ of **CL89145F** per 10^6 cells.
- 5. Vortex the tubes to ensure thorough mixing of antibody and cells.
- 6. Incubate the tubes for 30 minutes at 4°C.

 (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
- 7. Wash 2 times at 4°C.
- 8. Resuspend the cell pellet in 50 µl ice cold media B.
- 9. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

- A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + so-dium azide (100 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100μ) of 2M sodium azide in 100μ).

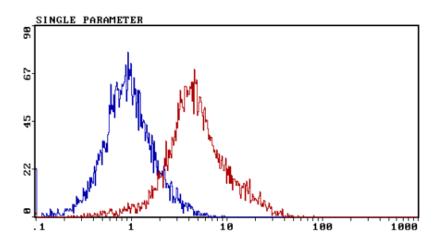
RESULTS:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

Cell Concentration: 1x10⁶ cells per test

Antibody Concentration Used: 1.0 µg/10⁶ cells Isotypic Control: FITC Rat IgG_{2a} (CLCR2a01)



CL89145FITC

Cell Source: Bone Marrow Dendritic Cells Percentage of cells stained above control: 49.0%

N.B. Appropriate control samples should always be included in any labeling studies.

* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.

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