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Affinity Purified Anti-Mouse Dendritic Cells (Cell Surface)

CL89145
Lot: 145210

DESCRIPTION:
CL89145 (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:
0.5 ml (containing 0.15 M PBS pH = 7.2), Lyophilized. 0.02% Sodium Azide is added as a preservative. BSA is added at 10 mg/ml as a stabilizer. Reconstitute in 0.5 ml of distilled water (stock solution). Minimum number of tests is 100. Concentration is 100 µg/ml.

SPECIFICITY:
Reacts with interdigitating cells, dendritic cells.

CLONE: NLDC-145

ISOTYPE: Rat IgG_{2a}

STORAGE/STABILITY:
Aliquots of stock solution can be kept frozen at -70°C. DO NOT FREEZE WORKING DILUTIONS. Stock solution stable for one year at -70°C. If not reconstituted ,stable for 1 year at 4°C.

WORKING DILUTION: A
Approximately 1:50-1:100 freshly prepared (Optimal concentration should be tested by serial dilutions.)

PROCEDURE FOR STAINING MOUSE TISSUE SECTIONS WITH CL89145:
1. Dry acetone fixed tissue sections (stored at -70°C) under air stream for 30 mins.
2. Incubate for 20 mins at room temperature in physiological phosphate buffered saline (PBS) containing 0.15M NaN_3 and add 500 ml H_2O_2 per 100 ml solution to block endogenous peroxidase activity.

Continued Overleaf…..

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3. Wash 3 x 3 mins. in PBS.
4. Incubate for 30 mins. at room temperature in a humid chamber in PBS containing 10% normal serum of the same species as second antibody used (**CL1200**) to avoid unspecific binding of detection reagent.
5. Wash 3 x 3 mins. in PBS.
6. Incubate for 1 hour with first antibody stock solution diluted in PBS for 1 hour at room temperature, or at 4^{o}C overnight, in a humid chamber.
7. Wash 3 x 3 mins. PBS.
8. Incubate for 1 hour at room temperature in a humid chamber with peroxidase conjugated goat anti-rat IgG (H+L) (**CLCC40007**) diluted in PBS containing 10% normal goat serum (**CL1200**) and 5% normal mouse serum (**CL8000**).
9. Wash 3 x 3 mins. in PBS.
10. Incubate for 12 min. at room temperature in the dark with substrate solution for peroxidase reaction. Add 100 ml of 0.1M acetate buffer pH 4.9 and 40 ml H_2O_2 to 5 ml of the stock solution AEC before use.

    Substrate: Stock solution of 0.4 g AEC=3-amino 9-ethylcarbazole in 100 ml dimethylformamide.

11. Wash 3 x 3 in PBS.
12. Counterstain with Mayers Hemalum.

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