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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

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FITC Anti-Mouse CD226 (DNAM-1) Monoclonal Antibody

CL8998F
CL8998F-3
LOT: 9831

DESCRIPTION:

CD226 (also called DNAM-1) is constitutively expressed on naive CD8+ T cells, as well as subsets of naive CD11b+ macrophages and NK cells. It is also found on a lower percentage (~40%) of unactivated CD4+ T cells. CD226 is functional upon T cell activation, and CD226 ligands (CD112 and CD155) are the same in the mouse as for human (Tage-4 is the mouse homologue of CD155). The gene for mouse DNAM-1 was identified on chromosome 18, and the predicted amino acid sequence shows a 53% homology with human DNAM-1.

Recent experiments show that this antibody (clone 15F.10E5) only binds to differentiated Th1 cells but not Th2 or Th0 cells. It also suppressed both antigen-specific T cell expansion and an autoimmune disease (EAE) mediated by effector Th1 cells.

This antibody is suitable for use in flow cytometry.

PRESENTATION:

100 µg (CL8998F) or 300 µg (CL8998F-3) 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.

SPECIFICATIONS:

Clone: 15F.10E5

Hybridoma Production:

Immunization: Immunogen: AE7 cells
Donor: Lewis rat

Fusion Partner: SP 2/0 myeloma

Specificity: Mouse CD226 (DNAM-1)

Ig Class: Rat IgM_k

Continued Overleaf...

For more information or to place an order please contact...

CEDARLANE®
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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.

Antibody Concentration: 0.1 mg/ml

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 2×10^7 cells/ml in media A. Add 50 μ 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 μ g of **CL8998F** or **CL8998F-3** 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 μ l 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 + sodium 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 (100 μ l of 2M sodium azide in 100 mls).

Results:

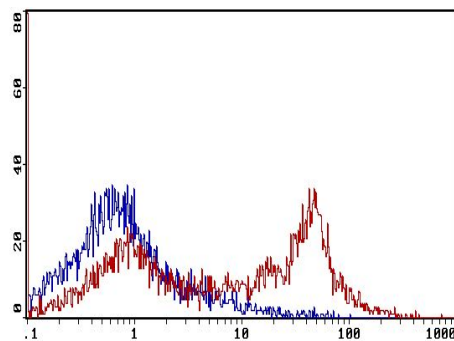
Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: Balb/C

Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: 1.0 μ g/ 10^6 cells

Isotypic Control: FITC Rat IgM (CLCRGM01)



Cell Source: Spleen
Percentage of cells stained above control: 26.4%

**N.B. Appropriate control samples should always be included in any labelling studies.
* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

Continued Overleaf....

REFERENCES:

1. Dardalhon, V., *et al.* 2005. CD226 is specifically expressed on the surface of Th1 cells and regulates their expansion and effector functions. *The Journal of Immunology*, **175**: 1558-1565.
2. Tahara-Hanaoka, S., *et al.* 2005. Identification and characterization of murine DNAM-1 (CD226) and its poliovirus receptor family ligands. *Biochemical and Biophysical Research Communications*, **329**: 996-1000.

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