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Product datasheet for AM31874PU-N

Cd226 Rat Monoclonal Antibody [Clone ID: 15F.10E5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	15F.10E5
Applications:	FC
Recommended Dilution:	Flow Cytometry.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgM
Clonality:	Monoclonal
Immunogen:	AE7 cells Donor: Lewis rat Fusion Partner: SP 2/0 myeloma
Specificity:	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.
Formulation:	PBS containing 0.02% sodium azide (NaN3) as preservative State: Purified State: Liquid purified Ig fraction
Concentration:	lot specific
Purification:	Affinity chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD226 antigen
Database Link:	Entrez Gene 225825 Mouse Q8K4F0

View online »

Background: CD226 (also called DNAM-1) is constituitively expressed on naive CD8+ T cells, as well as subsets of naïve 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.

Synonyms: DNAX accessory molecule 1, DNAM-1

Note: Protocol: **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.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.5 µg of this antibody.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (PE Goat anti-rat IgG (H+L)) at a 1/500 dilution
9. Incubate the tubes at 4°C for 30-60 minutes.

(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).

10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl 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 µl of 2M sodium azide in 100 mls).

B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: Balb/C

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.5 µg/10e6 cells

Isotypic Control: Rat IgG