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Product datasheet for CL032

Sell Rat Monoclonal Antibody [Clone ID: MEL-14]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MEL-14
Applications:	FC, FN, IHC, IP
Recommended Dilution:	Flow cytometry.3,4,5 Immunohistochemistry.4 Immunoprecipitation.3,5,6
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Mouse B cell Lymphoma, 38C-14 Donor: Fischer Rat Spleen Fusion Partner: P3 X 63Ag8.653
Specificity:	This monoclonal antibody reacts with a 90 kDa protein which is involved with the homing of lymphocytes to peripheral lymph nodes.
Formulation:	State: Ascites State: Lyophilized ascites
Reconstitution Method:	Restore with 0.5 ml of cold distilled water.
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C or -20°C. Following reconstitution store at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	selectin, lymphocyte
Database Link:	Entrez Gene 20343 Mouse P18337

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Background: L-selectin is expressed on most T and B lymphocytes, neutrophils, monocytes, eosinophils.1 Pre-incubation of lymphocytes with this antibody completely and specifically blocks binding of lymphocytes to high endothelial venules (HEV) in vitro2,3,6 and the migration of lymphocytes to lymph nodes in vivo.2,3 Polymorphonuclear cells preincubated with this antibody do not migrate to the inflammatory foci.

Synonyms: SELL, LNHR, LYAM1, Leu-8, TQ1, gp90-MEL, LECAM1, LAM-1

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:
1. Prepare a cell suspension in media A. For spleen 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 50 µl of a 1/20,000 - 1/50,000 dilution* of this Ab.
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 (FITC Goat anti-rat IgG (H+L)) at 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.

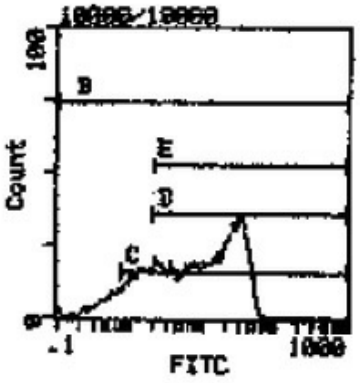
Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results - Tissue Distribution:
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 1/32,000 in 50 µl/10e6 cells
Isotypic Control: Rat IgG2a

Cell Source: Percentage of cells stained above control
Thymus: 64.1%
Spleen: 65.2%
Lymph Node: 75.1%

Results - Strain Distribution:
Tissue: Spleen
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 1/5000 in 50 µl
Strains Tested: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Positive: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Negative: none

Product images:



Cell Source: Lymph Node
Percentage of cells stained above control: 75.1%