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Product datasheet for SM268B

Cd28 Mouse Monoclonal Antibody [Clone ID: JJ319]

Product data:

Product Type:	Primary Antibodies
Clone Name:	JJ319
Applications:	FC
Recommended Dilution:	**Flow Cytometry** (for details please see "Protocols" below).
Reactivity:	Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Rat CD28 transfected A20J cells
Specificity:	Antibody SM268B recognizes a 90 kDa homeodimeric cell surface glycoprotein (CD28). **Results of tissue Distribution by Flow Cytometry Analysis:** Rat strain: Fischer Cell concentration: 1x10e6 cells per test Antibody concentration used: 1.0 µg/10e6 cells Isotypic control: Biotin Mouse IgG1 Cell source percentage of cells stained above control Thymus: 25.4% Splenic T Cells*: 73.2% *(T cells isolated with a Rat T Cell Recovery Column).
Formulation:	PBS containing 0.02% Sodium Azide and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml Label: Biotin State: Liquid purified Ig fraction
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Biotin
Storage:	Store at 2-8°C for up to one month. For longer storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.

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Stability: Shelf life: one year from despatch.

Database Link: Entrez Gene 25660 Rat
P31042

Background: CD28 has been found to be a potent costimulatory receptor on T cells. It is expressed on all peripheral rat alpha/beta and most gamma/delta T cells, as well as on approximately half of all NK cells.

Synonyms: TP44

Note: This clone can costimulate T cell proliferation and IL-2 secretion by resting rat T cells.

Protocol: **FLOW CYTOMETRY ANALYSIS:**

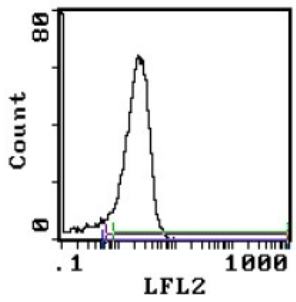
Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population.
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 1 x 10e6 cells, representing 1 test).
4. To each tube, add ~ 1.0 µg (optimize according to your assay conditions) of SM268B or SM268BX per 10e6 cells.
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 (Streptavidin-PE) at a appropriate dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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).

Product images:



Flow cytometry analysis

Cell Source: Splenic T Cells
Percentage of cells stained above control: 73.2%