

## Product datasheet for **SM268BX**

### Cd28 Mouse Monoclonal Antibody [Clone ID: JJ319]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	JJ319
Applications:	FC
Recommended Dilution:	<b>Flow Cytometry</b> (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). <b>Results of tissue Distribution by Flow Cytometry Analysis:</b> Rat strain: Fischer Cell concentration: 1x10e6 cells per test Antibody concentration used: 1.0 µg/10e6 cells Isotypic control: Biotin Mouse IgG1 <u>Cell source percentage of cells stained above control</u> 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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<b>Stability:</b>	Shelf life: one year from despatch.
<b>Gene Name:</b>	Cd28 molecule
<b>Database Link:</b>	<a href="#">Entrez Gene 25660 Rat P31042</a>
<b>Background:</b>	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.
<b>Synonyms:</b>	TP44
<b>Note:</b>	This clone can costimulate T cell proliferation and IL-2 secretion by resting rat T cells.

Protocol: **FLOW CYTOMETRY ANALYSIS:**

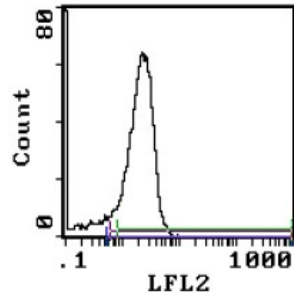
**Method:**

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  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add ~ 1.0  $\mu$ g (optimize according to your assay conditions) of SM268B or SM268BX 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.
7. Wash 2 times at 4°C.
8. Add 100  $\mu$ l 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  $\mu$ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l 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%