

Product datasheet for SM311A

OriGene Technologies, Inc.

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MHC Class I (RT1Aa) Mouse Monoclonal Antibody [Clone ID: OX-18]

Product data:

Product Type: Primary Antibodies

Clone Name: OX-18

Applications: FC, IHC, IP

Recommended Dilution: Flow Cytometry (see Protocols)

Reactivity: Rat

Host: Mouse Isotype: IgG1

Clonality: Monoclonal

Immunogen: Rat spleen membrane glycoproteins depleted of Ia-A antigens.

Donor: BALB/c spleen.

Fusion Partner: X63 Ag8.653.

Specificity: This monoclonal antibody recognizes a monomorphic determinant of rat Class I MHC antigen

(RT1.A) and thus reacts with all rat strains tested including AO(RT1µ), DA (RT1a), LEW (RT11) and PVG (RT1c). However, quantitiative measurements suggest that only a subfraction of the total Class I molecules are recognized (2). This antibody labels all peripheral lymphocytes but only a subfraction of thymocytes. It may be that the weakly labelled cells do in fact express some OX-18 antigenic determinants but there is clearly a major quantitative difference amongst thymocytes. This monoclonal used in tissue sections preferentially labels lymphoid cells in the medulla of the thymus including those cells with the marker phenotype of mature

T lymphocytes.

Formulation: PBS

State: Azide Free

State: Liquid pruified Ig

Concentration: lot specific

Purification: Protein G Chromotraphy

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. Handle under aseptic conditions.





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Stability: Shelf life: one year from despatch.

Database Link: P16391

Background: MHC Class I molecules play a central role in the immune system by presenting peptides

derived from the endoplasmic reticulum lumen. MHC class I antigens are heterodimers consisting of one alpha chain (44kDa) with beta 2 microglobulin (11.5 kDa). The antigen is expressed by all somatic cells at varying levels. MHC Class I molecules are expressed on most nucleated cells where they present endogenously synthesized antigenic peptides to CD8+ T lymphocytes, which are usually cytotoxic T cells. Fibroblasts or neurons however only show a

low level of antigen.



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®-Rat cell separation medium.
- 2. Wash 2 times.
- 3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
- 4. To each tube, add 0.2-0.5 µg* 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 (Goat anti-mouse IgG (H+L)-FITC conjugate) at 1:500 dilution.
- 9. Incubate 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.
- 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 μ l of 2M sodium azide in 100 mls).

Results - Tissue Distribution:

Rat Strain: Wistar

<u>Cell Concentration</u>: 1x10e6 cells per test

Antibody Concentration Used: 0.2 µg/10e6 cells

<u>Isotypic Control</u>: Mouse IgG1,k

Cell Source Percentage of cells stained above control:

Thymus: 38.1% Spleen: 97.0% Lymph Node: 99.9%

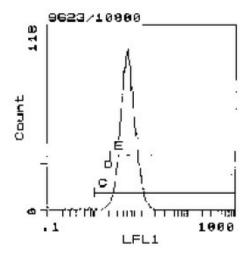
Results - Strain Distribution:

Antibody Concentration Used: 0.2 µg/10e6 cells Strains Tested: Wistar, Buffalo, Fischer 344 Positive: Wistar, Buffalo, Fischer 344

Negative: None



Product images:



Cell Source: Spleen - Percentage of cells stained above control: 97.0%