

Product datasheet for **CL129B**

MHC Class I (RT1Ac) Mouse Monoclonal Antibody [Clone ID: OX-27]

Product data:

Product Type:	Primary Antibodies
Clone Name:	OX-27
Applications:	FC, IHC
Recommended Dilution:	Flow Cytometry. Immunohistochemistry using cryostat sections: (however, cross-reactivity with Lewis rats have been shown to occur in some instances).
Reactivity:	Rat
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Phytohaemagglutinin Blasts Donor: BALB/c spleen Fusion Partner: P3-NS1-1-Ag4 (NS1/1)
Specificity:	This Antibody recognizes a polymorphic determinant of the MHC class I antigen in the rat. (c and n haplotypes). This antibody is an excellent antibody for labelling cells of donor or host origin in bone marrow chimeras.
Formulation:	PBS, 0.02% NaN ₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml Label: Biotin State: Liquid purified Ig
Concentration:	lot specific
Purification:	Protein A Chromatography
Conjugation:	Biotin
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.
Database Link:	Q31255



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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.

Synonyms: RT1-A3

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 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 0.5-1.0 µg* of this Ab 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-FITC) at a 1:500 dilution. Not recommended for use with a PE secondary.
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).

Results - Tissue Distribution:

Rat Strain: Brown Norway

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 1.0 µg/10e6 cells

Isotypic Control: Biotin Mouse IgG2a

Cell Source Percentage of cells stained above control:

Thymus 65.4%
Spleen 98.9%
Lymph Node 100%

Results - Strain Distribution:

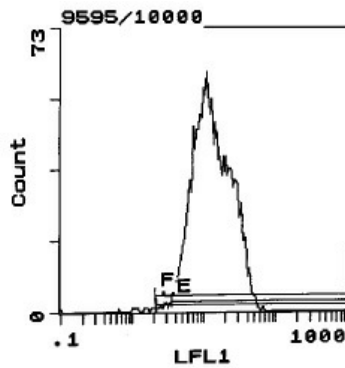
Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 1.0 µg/10e6 cells

Strains Tested: Brown Norway, Buffalo, Wistar, Fischer, ACI

Positive: Brown Norway

Negative: Buffalo, Wistar, Fischer, ACI

Product images:

Cell Source: Spleen

Percentage of cells stained above control: 98.9%