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Product datasheet for CL129P

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

Product data:

Product Type:	Primary Antibodies
Clone Name:	OX-27
Applications:	FC, IHC, IP
Recommended Dilution:	Flow Cytometry. Immunoprecipitation. 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 ecognizes 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 and 0.02% NaN3 State: Purified State: Liquid purified Ig
Concentration:	lot specific
Purification:	Protein G Chromatography
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.

Stability: Shelf life: one year from despatch.

Database Link: Q31255

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 1x10e6 cells, representing 1 test).
4. To each tube, add 0.5-2.0 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 (FITC Goat anti-mouse IgG (H+L)) at 1:500 dilution. Not recommended for use with PE secondary.
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:

Rat Strain: Brown Norway
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.5 µg/10e6 cells
Isotypic Control: Mouse IgG2a

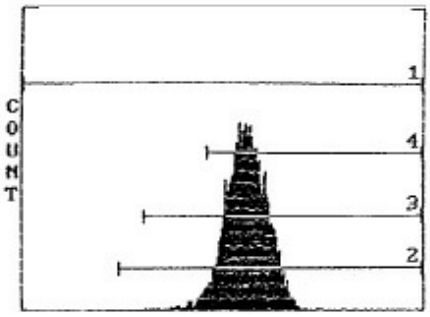
Cell Source Percentage of cells stained above control:

Thymus 39.8%
Spleen 95.9%
Lymph Node 99.9%

Results - Strain Distribution:

Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.5 µg/10e6 cells
Strains Tested: Lewis, Wistar, Brown Norway, Fischer 344, Buffalo, ACI
Positive: Brown Norway
Negative: Lewis, Wistar, Fischer 344 Buffalo, ACI

Product images:



LFL1
Cell Source: Spleen
Percentage of cells stained above control: 95.9 %