CL129FX
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Monoclonal Antibody to MHC Class I (RT1Ac) - FITC

Alternate names: MHC class I RT1.Ac heavy chain, RT1-A3

Catalog No.: CL129FX

Quantity: 0.5 mg

Concentration: 0.1 mg/ml

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.

Uniprot ID: Q31255

NCBI: 10116

Host / Isotype: Mouse / IgG2a

Clone: OX-27

Immunogen: Phytohaemagglutinin Blasts.

Format: **State:** Liquid purified IgG fraction.
Purification: Protein G Chromatography.
Buffer System: PBS buffer with 0.02% sodium azide as preservative and 0.5 % EIA grade BSA as stabilizer.
Label: FITC – conjugated

Applications: Flow cytometry: use 0.5 µg of neat antibody to label 10e6 cells.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Add. Information: Recognizes a polymorphic determinant of the MHC class I antigen in the rat.
This Antibody can be used for labelling cells of donor or host origin in bone marrow chimeras. (1,2)

Storage: Store the antibody at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References:
1. Butcher, G.W. (1987) 19: 3-21-Rat Membrane Alloantigens News.
2. Jeffries, W.A. et al. (1985) J. Exp. Med. 162: 117-127.

Protocols: **FLOW CYTOMETRY ANALYSIS:**

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with 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 0.5-0.2 µg antibody 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. (protect tubes from light)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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: 0.2 µg/10e6 cells
Isotypic Control: FITC Mouse IgG2a.

Cell Source-Percentage of cells stained above control:
Thymus: 73.9%
Spleen: 99.0%
Lymph Node: 100%