

Monoclonal Antibody to MHC Class I (RT1Ac) - FITC

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| Alternate names: | MHC class I RT1.Ac heavy chain, RT1-A3 |
| Catalog No.: | CL129F |
| Quantity: | 0.1 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: |

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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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.5-0.2 μ g antibody 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. (protect tubes from light)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 μ l ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 μ 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 μ l 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: Brown Norway
Cell Concentration: 1×10^6 cells per test.
Antibody Concentration Used: 0.2 μ g/ 10^6 cells
Isotypic Control: FITC Mouse IgG2a.

Cell Source-Percentage of cells stained above control:

Thymus: 73.9%
Spleen: 99.0%
Lymph Node: 100%

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