PE Anti-Mouse I-Ab
Monoclonal Antibody

CL8702PE
LOT: 8252

DESCRIPTION:
Cedarlane’s anti-mouse I-A\(^b\) monoclonal antibody reacts with the I-A\(^b\) encoded MHC class II antigen expressed on mouse strains of the H-2\(^b\) haplotype. It also reacts with the I-A\(^d\) encoded MHC class II antigen expressed on mouse strains of the H-2\(^d\) haplotype. Class II antigens are most highly expressed on antigen-presenting cells including B cells, macrophages, dendritic cells and certain epithelial cells.

PRESENTATION:
50 µg R-PE conjugated Ig buffered in PBS, 0.02% NaN\(_3\) and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE /STABILITY:
Store at 4°C. **DO NOT FREEZE**. Avoid prolonged exposure to light.

SPECIFICATIONS:
Clone: 28-16-8S
Specificity: Mouse MHC class II I-A\(^b\), cross reacts with I-A\(^d\)
Ig Class: Mouse IgM
Antibody Concentration: 0.1 mg/ml

Continued Overleaf......
FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10^7 cells/ml in media A. Add 50 μl of this suspension to each tube (each tube will then contain 1x10^6 cells, representing 1 test).
4. To each tube, add 0.2 μg of CL8702PE 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. (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive).
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 by Flow Cytometry Analysis:

Mouse strain: BALB/c
Cell Concentration: 1x10^6 cells per test
Antibody Concentration Used: 0.2 μg/10^6 cells
Isotypic Control: PE Mouse IgM (CLCMGM04)

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Percentage Stained Above Control</th>
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<tbody>
<tr>
<td>Thymus</td>
<td>28.2%</td>
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<tr>
<td>Spleen</td>
<td>40.7%</td>
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</tbody>
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N.B. Appropriate control samples should always be included in labelling studies.

* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.
REFERENCES: