PE Anti-Rat RT1.B<sup>µ</sup> Monoclonal Antibody

CL008PE
CL008PE-4
LOT: 0851

DESCRIPTION:

Cedarlane’s anti-rat RT1.B<sup>µ</sup> monoclonal antibody recognizes a polymorphic determinant on rat Ia present on Lewis, Wistar, and AO rat strains (although it is apparently expressed in lower amounts in AO rats) but not BN, ACI, DA, or PVG/c strains (1,2). This antibody also cross-reacts with mouse strains (MHC haplotypes b and s) and analysis of recombinant mouse strains shows that the determinant maps to the I-A region and correlates with mouse Ia specificity 9 (1). CL008PE recognizes Ia antigens on approximately 18% of thymocytes but does not significantly label the majority of peripheral T lymphocytes (1,3,4). Thus, CL008PE is particularly useful for distinguishing Ia positive cells from different rat strains and has been used in the recognition of cells of donor origin in bone marrow reconstituted radiation chimeras (3,4).

PRESENTATION:

50 µg (CL008PE) or 200 µg (CL008PE-4) R-PE conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> 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:** MRC OX-3

**Hybridoma Production:**

- **Immunization:** Immunogen: Rat thymocyte membrane glycoprotein
  Donor: BALB/c spleen

- **Fusion Partner:** P3-NS1-1-Ag4 (NS1/1)

**Specificity:** Rat RT1.B<sup>u</sup> (Rat Ia-polymorphic)

**Ig Class:** Mouse IgG<sub>1</sub>

**Format:** R-PE conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography)

**Antibody Concentration:** 0.1 mg/ml

**FLOW CYTOMETRY ANALYSIS:**

**Method:**

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte<sup>®</sup>-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10<sup>7</sup> cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10<sup>6</sup> cells, representing 1 test).
4. To each tube, add 0.05 µg* of **CL008PE or CL008PE-4** per 10<sup>6</sup> 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:**

Rat Strain: Buffalo
Cell Concentration: 1x10^6 cells per tests
Antibody Concentration Used: 0.05 µg/10^6 cells
Isotypic Control: PE Mouse IgG1

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Percentage of cells stained above control:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>18.6%</td>
</tr>
<tr>
<td>Spleen</td>
<td>43.9%</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>20.8%</td>
</tr>
</tbody>
</table>

N.B. Appropriate control samples should always be included in any labelling studies.
* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.

R-Phycoerythrin conjugates are produced under license and protected under Stanford University held patents 4,520,110; 4,542,104; 4,859,582; 5,055,556 (U.S.); 76695 (EPC); 548440 (Australia); 1,179,942 (Canada); and 1,594,827 (Japan).
REFERENCES:


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