PE Anti-Rat CD45
Monoclonal Antibody

CL001PE
CL001PE-4
LOT:0151

DESCRIPTION:
Cedarlane's anti-rat CD45 monoclonal antibody recognizes a monomorphic determinant of the rat leukocyte
common antigen.(1) The antigen recognized is a heavily glycosylated membrane glycoprotein of molecular weight
170,000 Da on thymocytes but molecular weight 170,000-220,000 Da on other leukocytes. The leukocyte common
antigen (L-CA) is a major glycoprotein of haematopoietic cells but is not found on other tissues or erythroid cells.
It is present on greater than 95% of thymocytes, bone marrow cells and thoracic duct lymphocytes. This molecule
carries much of the carbohydrate of thymocytes and shows interesting heterogeneity amongst T lymphocytes and B
lymphocytes. (2,3).

This antibody is suitable for use in flow cytometry.

PRESENTATION:
50 µg (CL001PE) or 200µg (CL001PE-4) conjugated Ig buffered in PBS, 0.02% NaN₃ 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-30
Hybridoma Production:
  Immunization: Immunogen: Lymph Node glycoproteins and cells
  Donor: BALB/c spleen
  Fusion Partner: NSI/1
Specificity: Rat CD45
Ig Class: Mouse IgG₂a
Format: R-PE conjugated Ig buffered in PBS, 0.02% NaN₃ 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

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®-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of $2 \times 10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1 \times 10^6$ cells, representing 1 test).
4. To each tube, 0.5 µg-1.0 µg of CL001PE or CL001PE-4 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:
Rat Strain: Wistar
Cell Concentration : $1 \times 10^6$ cells per test
Antibody Concentration Used: 0.5µg/$10^6$ cells
Isotopic Control: PE Mouse IgG2a (CLCMG2A04)

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Percentage of cells stained above control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>99.8%</td>
</tr>
<tr>
<td>Spleen</td>
<td>97.1%</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>98.9%</td>
</tr>
</tbody>
</table>

Cell Source: Spleen
Percentage of cells stained above control: 97.1%

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.

Strain Distribution by Flow Cytometry Analysis:
Procedure: see page 2
Strains Tested: Wistar, Buffalo, Brown Norway, Fischer 344
Positive: Wistar, Buffalo, Brown Norway, Fischer 344
Negative: none

Continued Overleaf….
REFERENCES: