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## Biotin Anti-Rat CD26 Monoclonal Antibody

CL061B  
CL061B-5  
LOT: 6141

### DESCRIPTION:

Cedarlane's anti-Rat CD26 monoclonal antibody (Clone: OX-61) recognizes and binds to the enzyme dipeptidyl peptidase IV (DPP-IV) present on rat thoracic duct lymphocytes and rat liver cells. The ectoenzyme DPP-IV is a bile canalicular cell surface molecule that has widespread tissue distribution. DPP-IV activity is specifically depleted from solubilized liver homogenates by CD26 bound to Sepharose. Under reducing conditions, the molecular weight of CD26 is 110kD. This antibody is the rat equivalent of human CD26. The N-terminal amino acid sequence of CD26 was sequenced and indicated identity with part of the predicted N-terminus of the bile canalicular molecule GP110. The cloning of DPP-IV permitted a comparison between the molecule recognized by monoclonal antibody CD26, GP110, and DPP-IV. It has been concluded that these three molecules are almost certainly identical.

This antibody is suitable for use in flow cytometry. This clone has also been reported to work in immunohistochemistry (cryostat sections), Western Blotting and immunoprecipitation.

### PRESENTATION:

100 µg (CL061B) or 500µg (CL061B-5) Biotin 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. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.

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For more information or to place an order please contact...

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## **SPECIFICATIONS:**

Clone: OX-61

### Hybridoma Production:

Immunization:

Immunogen: Thoracic duct lymphocytes from a mesenteric lymphodectomised rat

Donor: BALB/c mouse spleen

Fusion Partner: P3-NSI/1 Ag4-1

Specificity: Rat CD26

Ig Class: Mouse IgG2a

Format: Biotin 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.

Antibody Concentration: 0.1 mg/ml

## **FLOW CYTOMETRY ANALYSIS:**

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte<sup>®</sup>-R cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend cells to  $1 \times 10^6$  cells in approximately 50  $\mu$ l Media A in a microcentrifuge tube (i.e. 50  $\mu$ l of cells resuspended to  $2 \times 10^7$  cells/ml).  
(the contents of 1 tube represent 1 test).
4. To each tube add 2.0  $\mu$ g\* of **CL061B** or **CL061B-5** 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.
7. Wash 2 times at 4°C.
8. Add 100  $\mu$ l of secondary detection reagent **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in Media B.
11. Resuspend the cell pellet in 50  $\mu$ l ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l of propidium iodide at 0.5 mg/ml in phosphate buffered saline. (This stains dead cells by intercalating DNA).

### Media:

- A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

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Results:

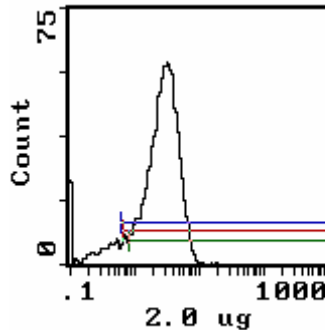
Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: FISHER

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration Used: 2.0  $\mu\text{g}/10^6$  cells

Isotypic Control: Biotin Mouse IgG2a (CLCMG2A15)



Percentage of cells stained above control: 89.5%

**N.B. Appropriate control samples should always be included in any labeling studies.**

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

**REFERENCES:**

1. McCaughan, G.W., Wickson, J.E., Creswick, P.F., Gorrell, M.D., 1990. Identification of the Bile Canalicular Cell Surface Molecule CP110 as the Ectopeptidase Dipeptidyl Peptidase IV: An Analysis by Tissue Distribution, Purification and N-Terminal Amino Acid Sequence. *Hepatology*; 11:534
2. Sandhu, J.S., Petkov, P.M., Dabeva, M.D., Shafritz, D.A. 2001. Stem Cell Properties and Repopulation of the Rat Liver by Fetal Liver Epithelial Progenitor Cells. *Am. J. Pathol.* 159: 1323-1334.
3. Cohen, D., Boulan, E., Musch, A. 2004. Par-1 promotes a hepatic mode of apical protein trafficking in MDCK cells. *PNAS.* 101(38): 13792-13797.

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