

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse LOX-1/OLR1: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 214012

Isotype: rat IgG_{2A}

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing LOX-1/OLR1 within a population and qualitatively determine the density of LOX-1/OLR1 on cell surfaces by flow cytometry.

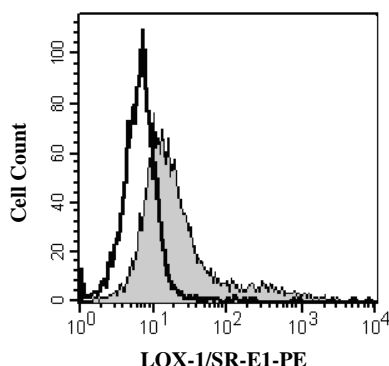
Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing LOX-1/OLR1. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing LOX-1/OLR1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of LOX-1/OLR1. Cell surface expression of LOX-1/OLR1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated rat anti-mouse LOX-1/OLR1:

Use as is; no preparation necessary.



RAW264.7 cells were stained with PE-conjugated anti-mouse LOX-1/OLR1 (Catalog # FAB1564P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of mouse IgG/10⁵ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated LOX-1/OLR1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted LOX-1/OLR1 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

Lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1), also known as oxidized low-density-lipoprotein receptor-1 (OLR-1), is a type II transmembrane receptor belonging to the C-type lectin family.¹ It also belongs to the functionally defined scavenger receptor (SR) superfamily, whose members share the common ability to bind and internalize modified forms of Low Density Lipoproteins (LDL).²⁻⁴ LOX-1 is the first member of the class E scavenger receptor subfamily (SR-E). It binds and supports the internalization of multiple structurally unrelated macromolecules including oxidized LDL, advanced glycation end products (AGE), activated platelets, bacteria, apoptotic or aged cells, and heat shock proteins.⁵⁻⁷ Recently, LOX-1 has also been implicated as an intestinal receptor involved in the transcytosis of pancreatic bile salt-dependent lipase.⁸ The mouse LOX-1 gene encodes a 363 amino acid residues (aa) protein with a short N-terminal intracellular domain, a transmembrane domain, triple repeats of an extracellular stalk/neck region followed by a C-type lectin-like domain (CTLN).¹¹ The CTLN, which is required for ligand recognition, contains the six conserved cysteine residues present in all C-type lectins, but lacks the Ca²⁺-binding residues found in classical C-type lectins. LOX-1 can be detected on activated endothelial cells, vascular smooth muscle cells, macrophages, intestinal cells and dendritic cells.⁵⁻⁸ The expression of LOX-1 is induced by proinflammatory or proatherogenic stimuli, as well as by oxidized LDL itself and hemodynamic or oxidative stress. LOX-1 exists on the cell surface as covalent homodimers, which can further associate into non-covalent-linked oligomers.⁹ Cell surface LOX-1 can also be cleaved by yet unidentified proteases to release the soluble LOX-1 extracellular domain.⁶ Binding and endocytosis of oxidized LDL by LOX-1 induces oxidative stress, activates NFκB, and upregulates the expression of monocyte chemoattractant protein-1 and matrix metalloproteinases.⁵⁻⁹ LOX-1-dependent oxidized LDL uptake also induces apoptosis by inducing the expression of the pro-apoptotic Bax and downregulation of the anti-apoptotic Bcl-2.¹⁰ Oxidized LDL plays a key role in the pathogenesis of atherosclerosis and endothelial dysfunction. Blockade of LOX-1 functions may turn out to be a suitable target for the therapeutic intervention of atherosclerosis.

References

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Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.