

Reagents Provided

Carboxyfluorescein (CFS)-conjugated rat monoclonal anti-human CD36/SR-B3: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 255606

Isotype: rat IgG_{2B}

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

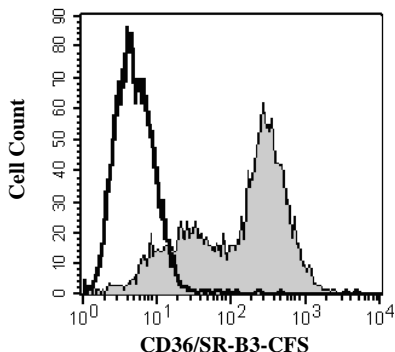
Designed to quantitatively determine the percentage of cells bearing CD36/SR-B3 within a population and qualitatively determine the density of CD36/SR-B3 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing CD36/SR-B3. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing CD36/SR-B3 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD36/SR-B3. Cell surface expression of CD36/SR-B3 is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.

Reagent Preparation

Fluorescein-conjugated rat anti-human CD36/SR-B3: Use as is; no preparation necessary.



HepG2 cells were stained with CFS-conjugated anti-human CD36/SR-B3 (Catalog # FAB19551F, filled histogram) or isotype control (Catalog # IC013F, 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) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred 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) 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 their substrates. Cells that require trypsinization to enable removal from their substrates 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 human 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 CFS-conjugated CD36/SR-B3 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CD36/SR-B3 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 CFS-labeled rat IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

CD36, alternatively known as platelet membrane glycoprotein IV (GPiV), GPIIb, thrombospondin receptor, collagen receptor, fatty acid translocase (FAT), and scavenger receptor class B, member 3 (SR-B3), is an integral membrane glycoprotein that has multiple physiological functions.¹ It is broadly expressed on a variety of cell types including microvascular endothelium, adipocytes, skeletal muscle, epithelial cells of the retina, breast, and intestine, smooth muscle cells, erythroid precursors, platelets, megakaryocytes, dendritic cells, monocytes/macrophages, and microglia.^{1,2} As a member of the scavenger receptor family, CD36 is a multi-ligand pattern recognition receptor that interacts with a large number of structurally dissimilar ligands, including long chain fatty acid (LCFA), advanced glycation end products (AGE), thrombospondin-1, oxidized low-density lipoproteins (oxLDLs), high density lipoprotein (HDL), phosphatidylserine, apoptotic cells, β -amyloid fibrils ($fA\beta$), collagens I and IV, and *Plasmodium falciparum*-infected erythrocytes.³ CD36 is required for the anti-angiogenic effects of thrombospondin-1 in the corneal neovascularization assay.⁴ It plays a role in lipid metabolism and has been identified as a fatty acid translocase necessary for the binding and transport of LCFA in cells and tissues.⁵ CD36 has been implicated in the clearance of apoptotic cells and cell debris and has also been shown to mediate the internalization and degradation of a variety of its ligands such as oxLDL, AGE and $fA\beta$.³ Upon ligand binding, CD36 transduces signals that mediate a wide range of pro-inflammatory cellular responses.² CD36 plays a significant role in the initiation and pathogenesis of chronic inflammatory diseases such as Alzheimer's disease and atherosclerosis.^{2,3} The human CD36 gene encodes a single-chain 472 amino acid residue protein containing both an N- and a C-terminal cytoplasmic tail and an extracellular loop.

References

1. Febbraio, M. *et al.*, 2001, *J. Clin. Invest.* **108**:785 - 791.
2. Khoury, J. *et al.*, 2003, *J. Exp. Med.* **197**:1657 - 1666.
3. Husemann, J. *et al.*, 2002, *Glia* **40**:195 - 205.
4. Armstrong, L and P. Bornstein, 2003, *Matrix. Biol.* **22**:63 - 71.
5. Febbraio M. *et al.*, 1999, *J. Biol. Chem.* **274**:19055 - 19062.

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.