

Reagents Provided

Carboxyfluorescein (CFS)-conjugated goat polyclonal anti-human IGF-II R: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

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

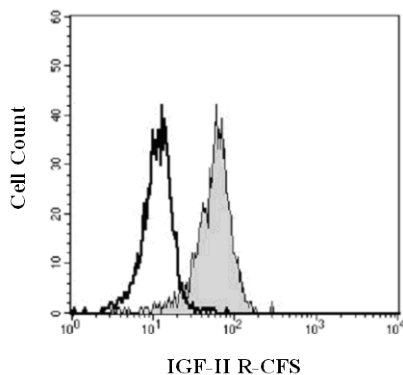
Designed to quantitatively determine the percentage of cells bearing IGF-II R within a population and qualitatively determine the density of IGF-II R on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled polyclonal antibody, which binds to cells expressing IGF-II R. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing IGF-II R are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IGF-II R. Cell surface expression of IGF-II R 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 goat anti-human IGF-II R: Use as is; no preparation necessary.



Human monocytes were stained with CFS-conjugated anti-human IGF-II R (Catalog # FAB2447F, filled histogram) or CFS-conjugated isotype control (Catalog # IC108F, 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 IGF-II R reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted IGF-II R reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysing 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 analysis by flow cytometry.
- 7) As a control for this analysis, cells in a separate tube should be treated with CFS-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The type 2 insulin-like growth factor receptor (also known as cation-independent mannose-6 phosphate receptor/CI-MPR) is a 300 kDa member of the P-type lectin family of molecules. P-type lectins generate functional eukaryotic lysosomes by binding and sorting lysosomal enzymes expressing phosphorylated mannose residues (M6P).¹⁻³ IGF-II R is a type I transmembrane glycoprotein that contains a 2,264 amino acid (aa) extracellular region, a 23 aa transmembrane segment and a 124 aa cytoplasmic tail.^{4,5} The extracellular region consists of 15 contiguous "binding" repeats of about 150 aa each. The odd-numbered repeats interact with "ligands", while the even-numbered repeats likely generate a non-disulfide homodimer in the membrane.¹ Repeat # 11 binds IGF-II, while repeats 3 and 9 bind mannose-6 phosphate; repeat #13 contains a fibronectin type II motif and assists in IGF-II binding.^{1,2} In the extracellular region of IGF-II R (600 aa's), human IGF-II R is 85% aa identical to both mouse and bovine IGF-II R. This region includes binding repeats # 11, 12, and 13. In addition to IGF-II, CI-MPR/IGF-II R binds non-M6P containing ligands such as retinoic acid, urokinase-type plasminogen-activator receptor and plasminogen, plus M6P-containing molecules such as lysosomal enzymes, TGF- β 1 precursor, proliferin, LIF, CD26, herpes simplex glycoprotein D and granzymes A and B.^{2,6} IGF-II R regulates many diverse biological functions that range from intracellular trafficking to the internalization of extracellular factors and modulation of cellular responses. It delivers newly synthesized M6P-tagged lysosomal enzymes from the trans-golgi network to endosomes, and facilitates the clearance of extracellular lysosomal and matrix degrading enzymes by internalization into clathrin-coated vesicles and delivery into endosomes. With respect to IGF-II biology, it appears that IGF-II R is principally a regulator of local IGF-II levels, targeting IGF-II for destruction in lysosomes.² However, some evidence suggests the receptor will signal via G-proteins, an effect that has yet to be conclusively shown.⁶

References

1. Ghosh, P. *et al.*, 2003, *Nat. Rev. Mol. Cell. Biol.* **4**:202.
2. Dahms, N.M. and M.K. Hancock, 2002, *Biochim. Biophys. Acta.* **1572**:317.
3. Zaina, S. and J. Nilsson, 2003, *Curr. Opin. Lipidol.* **14**:483.
4. Morgan, D.O. *et al.*, 1987, *Nature* **329**:301.
5. Oshima, A. *et al.*, 1988, *J. Biol. Chem.* **263**:2553.
6. Hawkes, C. and S. Kar, 2004, *Brain Res. Rev.* **44**:117.

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.