NEUROMICS

CXCR1/IL-8 RA Fluorescein Labeled Data Sheet

Catalog Number:	FC15001	**Species Reactivity:**	Human
Product Type	Mouse Monoclonal Protein G purified IgG_{2A} Antibody. Clone #: 42704	**Format:**	1.0 mL of fluorescein-labeled antibody at a concentration of 50 µg/mL.
Size:	100 Tests		
Intended Use:	Designed to determine the percentage of cells expressing the cell surface receptor CXCR1/IL-8 RA and the density of this receptor on cell surfaces by flow cytometry.	**Note:**	This reagent 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.
Storage:	2 -8° C		
Immunogen Sequence:	Clone #: 42704		

Application Notes

Additional Reagents Required
- PBS (Dulbecco's PBS)
- BSA

Principle of the Test
Washed cells are incubated with the FLUORESCEIN-labeled monoclonal antibody, which binds to cells expressing the CXCR1/IL-8 RA receptor. Unbound FLUORESCEIN-conjugated antibody is then washed from the cells. Cells expressing the CXCR1/IL-8 RA receptor are fluorescently stained, with the intensity of staining directly proportional to the density of CXCR1/IL-8 RA. Cell surface expression of the CXCR1/IL-8 RA receptor is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation
Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. 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. 50 µL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood cells will require RBC lysis 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 10^6 cells/mL and 25 µL of cells (1 x 10^5) 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 for 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 a rocker platform will prevent reattachment to the substrate.

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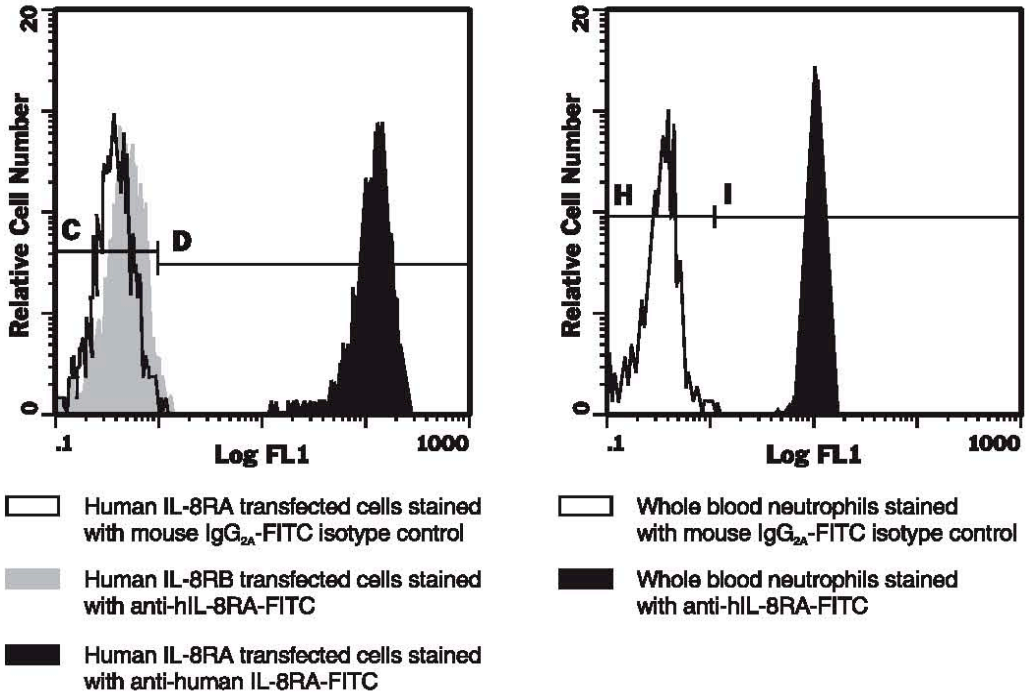
Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of FLUORESCEIN-conjugated anti-CXCR1/IL-8 RA reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CXCR1/IL-8 RA reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent).*
6) 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 FLUORESCEIN-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending upon final utilization.

Typical CXCR1/IL-8 RA Antibody Staining



(Left Panel) Typical staining observed with FAB330F on mouse cells transfected with the human CXCR1/IL-8 RA and CXCR2/IL-8 RB gene. An isotype control staining of the CXCR1 transfected cells is also shown.

(Right Panel) Typical staining observed with FC15001 on human peripheral granulocytes. An isotyFluorescein-matched conter staining of the same granulocytes is also shown.

References

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5. Horuk, R. *et al.* (1993) Biochemisry **32**:5733

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