



Catalog Number:	FC15004	Species Reactivity:	Human
Product Type	Mouse Monoclonal Protein G purified IgG _{2B} Antibody. Clone #: 44717	Format:	1.0 mL of PE-labeled antibody, at a concentration of 25 µg/mL.
Size:	100 Tests		
Intended Use:	Designed to determine the percentage of cells expressing the cell surface receptor CXCR-4 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		

Application Notes and Protocol

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing the CXCR-4 receptor. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing the CXCR-4 receptor are fluorescently stained, with the intensity of staining directly proportional to the density of CXCR-4. Cell surface expression of the CXCR-4 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 4×10^6 cells/mL and 25 µL of cells (1×10^5) 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.

FOR RESEARCH USE ONLY

NEUROMICS' REAGENTS ARE FOR IN VITRO AND CERTAIN NON-HUMAN IN VIVO EXPERIMENTAL USE ONLY AND NOT INTENDED FOR USE IN ANY HUMAN CLINICAL INVESTIGATION, DIAGNOSIS, PROGNOSIS, OR TREATMENT. THE ABOVE ANALYSES ARE MERELY TYPICAL GUIDES. THEY ARE NOT TO BE CONSTRUED AS BEING SPECIFICATIONS. ALL OF THE ABOVE INFORMATION IS, TO THE BEST OF OUR KNOWLEDGE, TRUE AND ACCURATE. HOWEVER, SINCE THE CONDITIONS OF USE ARE BEYOND OUR CONTROL, ALL RECOMMENDATIONS OR SUGGESTIONS ARE MADE WITHOUT GUARANTEE, EXPRESS OR IMPLIED, ON OUR PART. WE DISCLAIM ALL LIABILITY IN CONNECTION WITH THE USE OF THE INFORMATION CONTAINED HEREIN OR OTHERWISE, AND ALL SUCH RISKS ARE ASSUMED BY THE USER. WE FURTHER EXPRESSLY DISCLAIM ALL WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.

02/07v1

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

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/10⁵ 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 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated anti-CXCR-4 reagent.
- 4) Incubate for 30 - 45 minutes at 2 - 8° C.
- 5) Following this incubation, remove unreacted anti-CXCR-4 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 PE-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending upon final utilization.

References

1. Bleul, C.C. *et al.* (1996) *Nature* **382**:829.
2. Oberlin, E. *et al.* (1996) *Nature* **382**:833.
3. Feng, Y. *et al.* (1996) *Science* **272**:872.
4. Endres, M.J. *et al.* (1996) *Cell* **87**:745.
5. Lu, Z.H. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**:6426.

FOR RESEARCH USE ONLY

NEUROMICS' REAGENTS ARE FOR IN VITRO AND CERTAIN NON-HUMAN IN VIVO EXPERIMENTAL USE ONLY AND NOT INTENDED FOR USE IN ANY HUMAN CLINICAL INVESTIGATION, DIAGNOSIS, PROGNOSIS, OR TREATMENT. THE ABOVE ANALYSES ARE MERELY TYPICAL GUIDES. THEY ARE NOT TO BE CONSTRUED AS BEING SPECIFICATIONS. ALL OF THE ABOVE INFORMATION IS, TO THE BEST OF OUR KNOWLEDGE, TRUE AND ACCURATE. HOWEVER, SINCE THE CONDITIONS OF USE ARE BEYOND OUR CONTROL, ALL RECOMMENDATIONS OR SUGGESTIONS ARE MADE WITHOUT GUARANTEE, EXPRESS OR IMPLIED, ON OUR PART. WE DISCLAIM ALL LIABILITY IN CONNECTION WITH THE USE OF THE INFORMATION CONTAINED HEREIN OR OTHERWISE, AND ALL SUCH RSKS ARE ASSUMED BY THE USER. WE FURTHER EXPRESSLY DISCLAIM ALL WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.

02/07v1

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics1.com