

## Reagents Provided

**Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human CXCR4:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 12G5

**Isotype:** mouse IgG<sub>2A</sub>

## 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

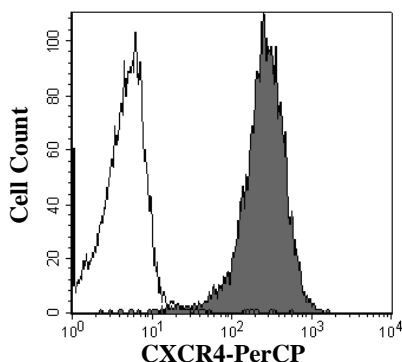
Designed to quantitatively determine the percentage of cells bearing CXCR4 within a population and qualitatively determine the density of CXCR4 on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the PerCP-labeled monoclonal antibody, which binds to cells expressing CXCR4. Unbound PerCP-conjugated antibody is then washed from the cells. Cells expressing CXCR4 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CXCR4. Cell surface expression of CXCR4 is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.

## Reagent Preparation

**PerCP-conjugated mouse anti-human CXCR4:** Use as is; no preparation necessary.



Jurkat cells were stained with PerCP-conjugated anti-human CXCR4 (Catalog # FAB170C, filled histogram) or isotype control (Catalog # IC003C, open histogram).

 フナコシ株式会社

試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775

e-mail: reagent@funakoshi.co.jp

2010/04/02

## 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 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) 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/10<sup>5</sup> 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 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PerCP-conjugated CXCR4 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CXCR4 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 analysis by flow cytometry.
- 7) As a control for analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

**R&D Systems Inc.**  
**1-800-343-7475**

## Background Information

CXCR4 (fusin or LESTR) is a G-protein-linked seven transmembrane spanning chemokine receptor that binds stromal cell-derived factor-1 (SDF-1).<sup>1,2</sup> CXCR4 acts as a co-factor for T-cell tropic HIV-1 and -2 viral entry into cells.<sup>3,4</sup> A monoclonal antibody (clone 12G5) to the CXCR4 structure was developed by Enders, *et al.*<sup>4</sup> By flow cytometry, clone 12G5 reacts with CXCR4 expressed on a variety of human cell lines, including Sup-T1, Hut-78, Molt4, CEMss, Daudi and Hela, as well as peripheral blood lymphocytes.<sup>4</sup> Clone 12G5 can also neutralize infection and inhibit syncytium formation induced by the HIV virus.<sup>4</sup> The epitope for the 12G5 monoclonal antibody has been mapped to a region of the CXCR4 receptor that includes extracellular loops 1 and 2 (extracellular loop 1 plays a more critical role in the overall epitope structure).<sup>5</sup>

## 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.

**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.