

## Reagents Provided

**Carboxyfluorescein (CFS)-conjugated goat polyclonal anti-mouse 2B4/CD244/SLAMF4:** Supplied as 50 µg of antibody in 1 mL PBS containing 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 2B4/CD244/SLAMF4 within a population and qualitatively determine the density of 2B4/CD244/SLAMF4 on cell surfaces by flow cytometry.

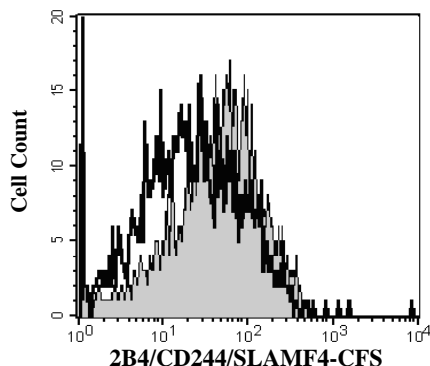
## Principle of the Test

Washed cells are incubated with the fluorescein-labeled polyclonal antibody, which binds to cells expressing 2B4/CD244/SLAMF4. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing 2B4/CD244/SLAMF4 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of 2B4/CD244/SLAMF4. Cell surface expression of 2B4/CD244/SLAMF4 is determined by flow cytometric analysis 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-mouse

**2B4/CD244/SLAMF4:** Use as is; no preparation necessary.



NK cells from mouse spleen were stained with CFS-conjugated anti-mouse 2B4/CD244/SLAMF4 (Catalog # FAB1050F, filled histogram) or isotype control (Catalog # IC108F, open histogram).

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## 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) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells 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), 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<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 substrate. Cells that require trypsinization to enable 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 the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of mouse 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 CFS-conjugated 2B4/CD244/SLAMF4 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted 2B4/CD244/SLAMF4 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 goat IgG antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

2B4, also known as CD244 and SLAMF4, is a 66 kDa type I transmembrane glycoprotein in the SLAM subgroup of the CD2 protein family. 2B4 interacts with CD48, while other SLAM family proteins interact homophilically.<sup>1-4</sup> 2B4 is expressed on all NK cells,  $\gamma\delta$  T cells, monocytes, some CD4+ and CD8+ T cells, and some dendritic cells.<sup>5</sup>

## References

1. Bhat, R. *et al.*, 2006, J. Leukocyte Biol. **79**:417.
2. Veillette, A., 2006, Nat. Rev. Immunol. **6**:56.
3. McNerney, M.E. *et al.*, 2005, Mol. Immunol. **42**:489.
4. Assarsson, E. *et al.*, 2005, J. Immunol. **175**:2045.
5. Nakajima, H. *et al.*, 1999, Eur. J. Immunol. **29**:1676.

**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.