

# Monoclonal Anti-human LAIR1-Fluorescein

Catalog Number: FAB2664F

Lot Number: AACW01

100 Tests

## Reagents Provided

**Carboxyfluorescein-conjugated mouse monoclonal anti-human LAIR1:** Contains 1.0 mL of CFS-labeled antibody at a concentration of 25 µg/mL.

**Clone #:** 342219

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

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

**All Reagents:** 2 - 8° C

## Intended Use

Designed to quantitatively determine the percentage of cells bearing LAIR1 within a population and qualitatively determine the density of LAIR1 on cell surfaces by flow cytometry.

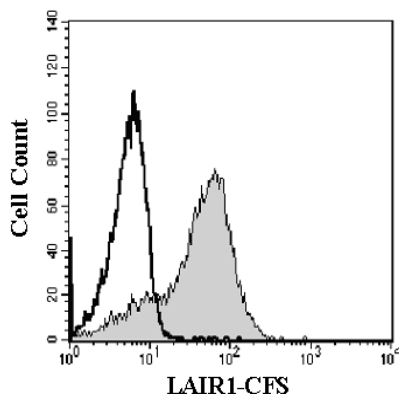
## Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing LAIR1. Unbound fluorescein -conjugated antibody is then washed from the cells. Cells expressing LAIR1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of LAIR1. Cell surface expression of LAIR1 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 mouse anti-human LAIR1:**

Use as is; no preparation necessary.



Human lymphocytes were stained with anti-human LAIR1 (R&D Systems, Cat. # FAB2664F, filled histogram) or isotype control (R&D Systems, Cat. # IC0041F, open histogram).

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 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 CFS-conjugated LAIR1 reagent.
- 4) Incubate for 30 - 45 minutes at 2 - 8° C.
- 5) Following this incubation, remove unreacted LAIR1 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 mouse IgG<sub>2b</sub> antibody.

This procedure may need modification, depending upon final utilization.

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**Background Information**

LAIR1 is an inhibitory receptor belonging to the Ig superfamily. It is a type I transmembrane protein with one extracellular Ig-like domain and two cytoplasmic ITIMs. Four LAIR1 splice variants exist. LAIR1b has a 17 aa deletion outside the Ig loop in the extracellular domain. It differs from LAIR1c by one aa residue. LAIR1d has a 77 aa truncation in the cytoplasmic domain. LAIR1 is expressed on NK cells, T cells, B cells, monocytes, dendritic cells and most thymocytes. The extracellular domain of human LAIR1 shares 40% aa identity with that of the mouse protein.

**Note:** *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.*