

## Reagents Provided

**Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human IL-1 $\beta$ /IL-1F2:** Supplied as 25  $\mu$ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 8516

**Isotype:** mouse IgG<sub>1</sub>

## Reagents Not Provided

**Flow Cytometry Fixation Buffer** (Catalog # FC004) or other 4% paraformaldehyde fixation buffer

**Flow Cytometry Permeabilization/Wash Buffer I (1X)** (Catalog # FC005) or other saponin-containing saline buffer.

## Storage

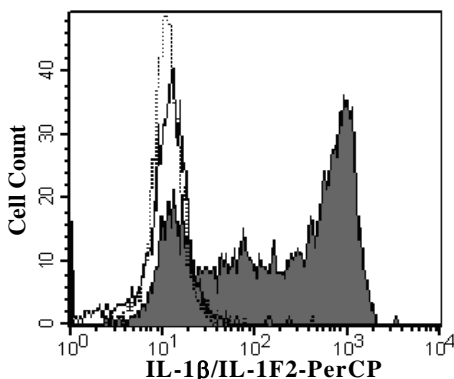
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells containing IL-1 $\beta$ /IL-1F2 within a population and qualitatively determine the density of intracellular IL-1 $\beta$ /IL-1F2 by flow cytometry.

## Product Description

Produced from a hybridoma elicited from a mouse immunized with purified, *E. coli*-derived, recombinant human IL-1 $\beta$  (rhIL-1 $\beta$ ; aa 117 - 269; Accession # P01584). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to a PerCP fluorochrome. Intracellular expression of IL-1 $\beta$ /IL-1F2 is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



PBMC monocytes, unstimulated (open histogram-solid line) or stimulated with LPS (filled histogram) were stained with PerCP-conjugated anti-human IL-1 $\beta$ /IL-1F2 (Catalog # IC201C) or isotype control (Catalog # IC002C, open histogram-dotted line).

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

IL-1 $\beta$ , also known as IL-1F2, is a prototypical member of the IL-1 superfamily. It is produced by a wide variety of cells in response to inflammatory agents, infections, or microbial endotoxins. The biological activity of IL-1 $\beta$  is mediated by the heterodimeric receptor complex containing IL-1 R1 (IL-1 RI) and IL-1 R3 (IL-1 RAcP). The IL-1 $\beta$  propeptide is cleaved intracellularly by caspase-1/ICE to generate a 17 kDa active cytokine. Mature human IL-1 $\beta$  shares 77% amino acid sequence identity with mouse and rat IL-1 $\beta$ .

## Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see [Reagents Not Provided](#)).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. 5 x 10<sup>5</sup> cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10  $\mu$ L of conjugated antibody was added and cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG<sub>1</sub> antibody. This procedure may need to be modified, depending on cell type and final utilization. Individual users may need to titrate to determine optimal reagent amount for their specific use.

**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.