Technical Data Sheet

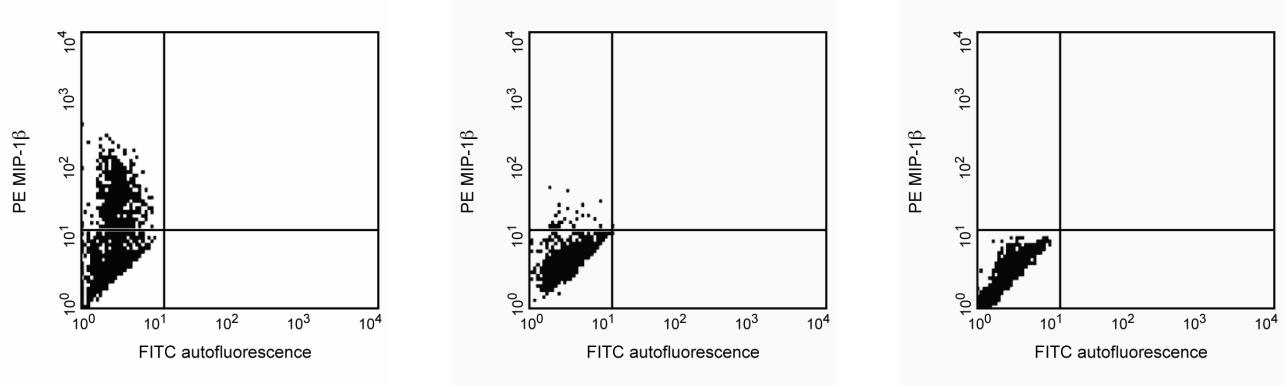
PE Mouse Anti-Human MIP-1β

Product Information

Material Number:	**561120**
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	D21-1351
Immunogen:	Recombinant Human MIP-1β
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The D21-1351 monoclonal antibody specifically binds to the human CC chemokine, MIP-1β (macrophage inflammatory protein-1β). Human MIP-1β shares approximately 75% homology with mouse MIP-1β at the amino acid level. Expression of MIP-1β in human peripheral blood cells is induced by proinflammatory and mitogenic stimuli. MIP-1β is a chemoattractant for monocytes and lymphocytes. Human MIP-1β binds to receptors, CCR5 and CCR8. The human MIP-1β gene has been mapped to chromosome 17q11. The immunogen used to generate D21-1351 hybridoma was recombinant human MIP-1β.



Expression of MIP-1β by stimulated human monocytes. *Human PBMC were stimulated with human IFN-γ (20 ng/ml final concentration; Cat. No. 554616/554617) for one hour followed by overnight stimulation with LPS (1 µg/ml final concentration; Sigma, Cat. No. L-8272) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, fixed, permeabilized, and stained with 0.03 µg of PE-mouse anti-human MIP-1β antibody (PE-D21-1351, Cat. 550078) following Pharmingen's staining protocol (see Figure, Left panel). The data reflects gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, binding by the PE- D21-1351 antibody was blocked by preincubation of the PE-D21-1351 antibody with recombinant human MIP-1β (0.25 µg; Middle panel) and by preincubation of the fixed/permeabilized cells with excess unlabeled D21- 1351 antibody (5 µg; Right panel) prior to staining with the PE-D21-1351 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control and verified using the unlabeled antibody blocking control.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

IF/Flow: For immunofluorescent staining and flow cytometric analysis, the D21-1351 antibody has been found useful to identify and enumerate MIP-1β producing cells within mixed cell populations. PE conjugated D21-1351 antibody (Cat. No. 550078) is especially suitable for these studies.

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A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponinpermeabilized human cells is PE-MOPC-21 immunoglobulin (Cat. No. 554680); use at comparable concentrations to antibody of interest (e.g., 0.5 µg mAb/ million cells). A useful control for demonstrating specificity of staining is to pre-block the fixed/permeabilized cells with unlabeled D21-1351 antibody, prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554617	Recombinant Human IFN-γ	50 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Bernardini G, Hedrick J, Sozzani S. Identification of the CC chemokines TARC and macrophage inflammatory protein-1 beta as novel functional ligands for the CCR8 receptor. *J Immunol.* 1998; 28(2):582-588. (Biology)

Combadiere C, Ahuja SK, Tiffany HL, Murphy PM. Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1(alpha), MIP-1(beta), and RANTES. *J Leukoc Biol.* 1996; 60(1):147-152. (Biology)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology: Flow cytometry, IC/FCM Block)

Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. *J Biol Chem.* 1996; 271(29):17161-17166. (Biology)