Technical Data Sheet

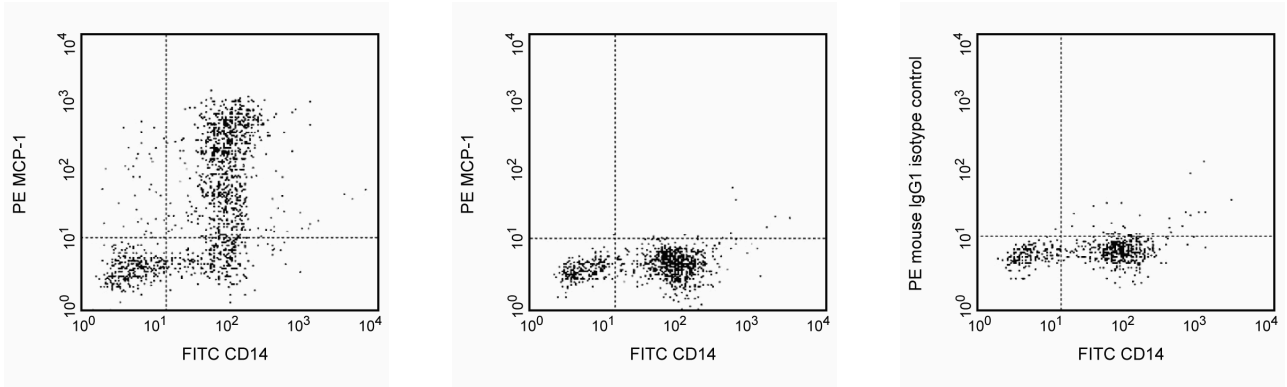
PE Mouse Anti-Human MCP-1

Product Information

Material Number:	**559324**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	5D3-F7
Immunogen:	Recombinant Human MCP-1
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 5D3-F7 antibody reacts with human monocyte chemoattractant protein-1 (MCP-1), also known as monocyte chemotactic and activating factor (MCAF).



Expression of MCP-1 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 24 hours with LPS (10 ng/ml final concentration) in the presence of BD GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC-mouse anti-human CD14 antibody (FITC-M5E2,Cat. No. 555397), fixed, permeabilized, and subsequently stained with 20 µl of PE-mouse anti-human MCP-1 antibody (PE-5D3-F7, Cat. No. 559324) by following the Usage section below and BD Biosciences Pharmingen's staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, binding by the PE-5D3-F7 antibody was blocked by preincubation of the fixed/permeabilized cells with excess unlabeled 5D3-F7 antibody (5 µg; Cat. No. 554662; middle panel) prior to staining with the PE-5D3-F7 antibody. The level of non-specific staining was assessed using the PE-mouse IgG1 isotype control (0.25 µg; PE-MOPC-21; Cat. No. 554680; right panel). 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 antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated 5D3-F7 antibody (Cat. No. 559324) can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate MCP-1-producing cells within mixed cell populations (see image). This 100 Test Size formulation of the PE-conjugated 5D3-F7 antibody has been pre-titrated to assure effective intracellular detection of human MCP-1 using 20 µl/ 1 x 10^6 cells.

BD Biosciences

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A useful control for demonstrating specificity of staining is to pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled 5D3-F7 antibody (Cat. No. 554662) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-MOPC-21 immunoglobulin (Cat. No. 559320). For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. BD Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the Usage section below.

Usage

1. Resuspend 1 × 10^6 fixed (BD Cytofix/Cytoperm™, Cat. No. 554722) and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1× BD Perm/Wash™ Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (at RT or 4°C).
3. Wash twice in 100 µl of 1× BD Perm/Wash™ Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554662	Purified Mouse Anti-Human MCP-1	80 tests	5D3-F7
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555397	FITC Mouse Anti-Human CD14	100 tests	M5E2
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Peri G, Milanese C, Matteucci C, et al. A new monoclonal antibody (5D3-F7) which recognizes human monocyte-chemotactic protein-1 but not related chemokines. Development of a sandwich ELISA and in situ detection of producing cells. *J Immunol Methods.* 1994; 174(1-2):249-257. (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. (Clone-specific: Flow cytometry)