Technical Data Sheet

PE Hamster Anti-Mouse/Rat MCP-1

Product Information

Material Number:	**554443**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	2H5
Immunogen:	Heparin-purified CHO-expressed mouse MCP-1
Isotype:	Armenian Hamster IgG1, κ
Reactivity:	QC Testing: Rat, Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2H5 antibody reacts with mouse and rat monocyte chemoattractant protein (MCP-1), formerly termed JE. This antibody also recognizes human MCP-1, but shows no reactivity with the closely related mouse β chemokines, TCA3 and MIP-1β. The immunogen used to generate the 2H5 hybridoma was heparin-purified CHO-expressed mouse MCP-1. This is a neutralizing antibody.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

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 by gel filtration chromatography.

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:

Immunofluorescent Staining: The 2H5 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate MCP-1 producing cells within mixed cell populations. For optimal immunofluorescent staining for flow cytometric analysis, the anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit the protocols sections, or the chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our website, www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is either of the following: 1) preblock the conjugated 2H5 antibody with a ligand (e.g., recombinant mouse MCP-1; Cat No. 554590 for mouse or recombinant rat MCP-1; Cat. No. 555110 for rat) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled 2H5 antibody (Cat. No. 554441) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable hamster IgG isotype control for assessing the level of background staining on fixed/permeabilized mouse cells is PE-G235-2356 (Cat. No. 554711); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

ELISA: Purified 2H5 antibody (Cat. No. 554441) can be used as the capture antibody in a sandwich ELISA for measuring mouse MCP-1 levels in conjunction with the biotinylated 4E2/MCP mouse antibody (Cat. No. 554444) as the detection antibody, and recombinant mouse MCP-1 (Cat. No. 554590) as the standard.

Neutralization: The NA/LE™ 2H5 antibody is useful for neutralization of mouse MCP-1 bioactivity.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554711	PE Hamster IgG1, λ1 Isotype Control	0.1 mg	G235-2356
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554654	Mick-3 Cytokine Positive Control Cells	5x10^6 cells	(none)

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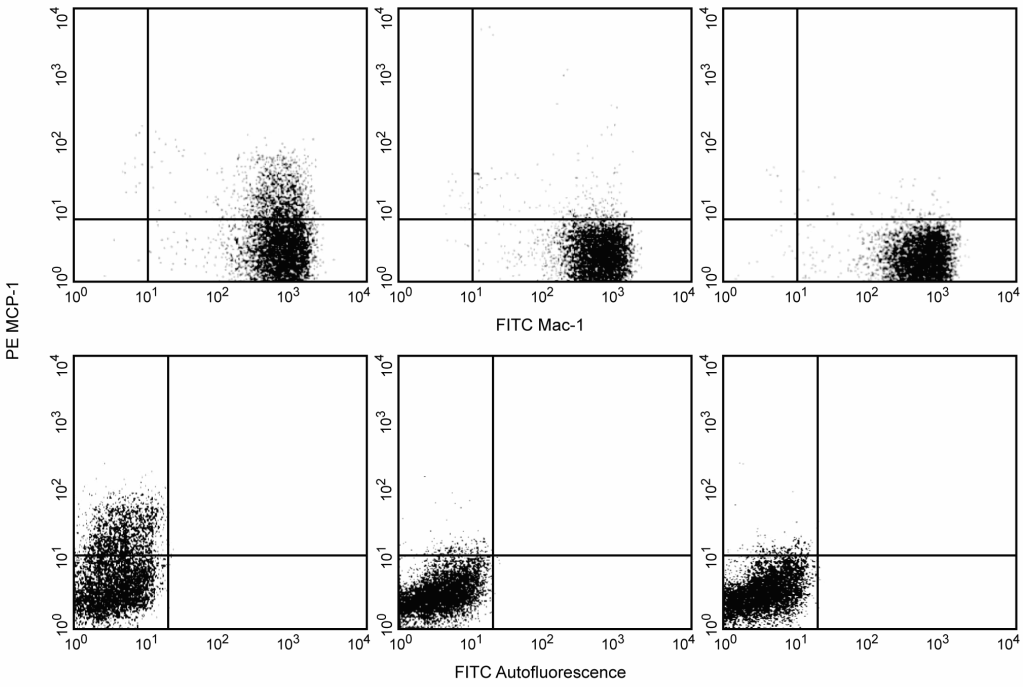

Expression of mouse (top row) or rat (bottom row) MCP-1 by stimulated peritoneal cells. Mouse*: Thioglycolate-elicited peritoneal macrophages from 6 month old BALB/c mice were stimulated with LPS (1 µg/ml; Sigma) for 5 hours in culture in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). Fc receptors were blocked using 0.5 µg of Fc Block™ (Cat. No. 553142). Cells were stained with 0.06 µg of FITC-rat anti-mouse Mac-1 antibody, fixed, permeabilized, and then stained with 0.25 µg of PE-hamster anti-mouse MCP-1 antibody (PE-2H5; Cat. No. 554443) by using Pharmingen's staining protocol. To demonstrate specificity of staining, the binding of PE-2H5 was blocked by the preincubation of the conjugated antibody with recombinant mouse MCP-1 (0.25 µg, Cat. No. 554590; middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled 2H5 antibody (8 µg, Cat. No. 554711; right panel) prior to staining with the PE-2H5 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) and unlabeled antibody blocking (right panel) specificity controls.* ***Rat****: Peritoneal cells from Lewis rats were harvested and plated in complete RPMI for one week. Cells were stimulated with LPS (1 µg/ml final concentration; Sigma) overnight in the presence of GolgiPlug™ (1 µg/ml; Cat. No. 555029), harvested and blocked for nonspecific staining with purified rat IgG. Cells were fixed, permeabilized, and then stained with 0.25 µg of PE-hamster anti-mouse MCP-1 antibody (PE-2H5; Cat. No. 554443). To demonstrate specificity of staining, the binding of PE-2H5 was blocked by the preincubation of the conjugated antibody with recombinant rat MCP-1 (0.25 µg, Cat. No. 555110; middle panel), and by preincubation of the fixed/ permeabilized cells with the unlabeled antibody blocking (right panel) specificity controls.*

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
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.
4. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
5. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

References

Luo Y, Laning J, Hayashi M, Hancock PR, Rollins B, Dorf ME. Serologic analysis of the mouse beta chemokine JE/monocyte chemoattractant protein-1. *J Immunol.* 1994; 153(8):3708-3716.(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)