Technical Data Sheet

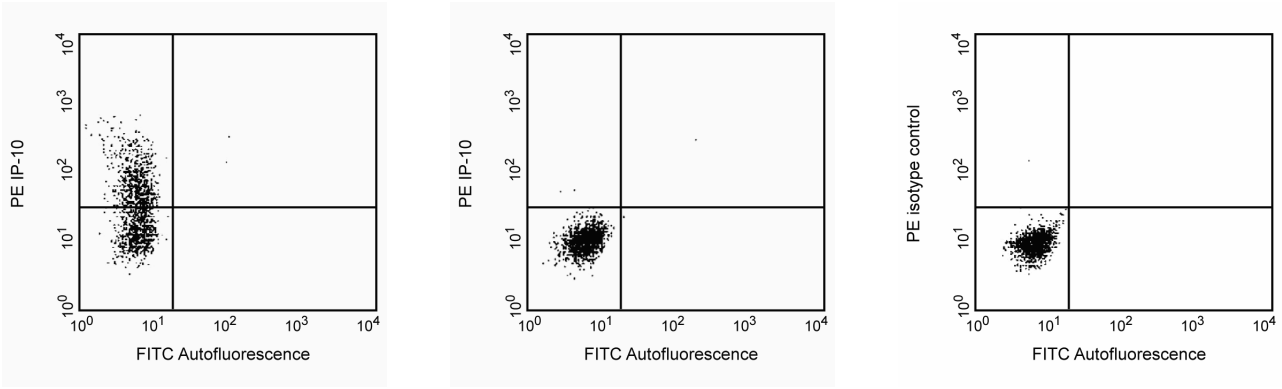
PE Mouse Anti-Human IP-10

Product Information

Material Number:	**555049**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	6D4/D6/G2
Immunogen:	Recombinant human IP-10 protein
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody 6D4/D6/G2 reacts with human CXC chemokine, interferon gamma inducible protein 10 (IP-10). IP-10 is inducible in monocytes, keratinocytes and endothelial cells by IFN-γ. The immunogen used to generate the monoclonal antibody 6D4/D6/G2 was recombinant human IP-10 protein.



Expression of IP-10 by stimulated human monocytes. *Human PBMC were stimulated for 24 hours with Human Interferon-γ (1500 U/ml final concentration; Cat. No. 554617) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, fixed, permeabilized, and stained with 0.05 µg of PE-mouse anti-human IP-10 antibody (PE-6D4/D6/G2, Cat. No. 555049) following BD Pharmingen staining protocol (see Figure, 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-6D4/D6/G2 antibody was blocked by preincubation of the fixed/permeabilized cells with excess unlabeled 6D4/D6/G2 antibody (5 µg; middle panel) prior to staining with the PE-6D4/D6/G2 antibody. The level of nonspecific staining was assessed using the PE-mouse IgG2a isotype control (0.05 µg; PE-G155-178; Cat. No. 554648; 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

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

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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated 6D4/D6/G2 antibody (Cat. No. 555049) can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate human IP-10-producing cells within mixed cell populations (See Figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated. (≤ 0.5 µg mAb/1X10^6 cells) For specific methodology, please see the online protocols or the chapter on intracellular staining in the Immune Function handbook posted at www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is to pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled 6D4/D6/G2 antibody prior to staining. The staining technique and use of blocking controls are described by C. Prussin, et al. A suitable mouse IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is

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PE-G155-178 (Cat. No. 554648); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1X10^6 cells).

ELISA: The biotinylated 6D4/D6/G2 (Cat. No. 555048) antibody is useful as a detection antibody for a sandwich ELISA for specifically measuring human IP-10 protein levels. Biotin 6D4/D6/G2 antibody can be paired with the Purified 4D5/A7/C5 antibody (Cat. No. 555046) as the capture antibody and with Recombinant human IP-10 protein (Cat. No. 551130) as the standard.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554648	PE Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/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. An isotype control should be used at the same concentration as the antibody of interest.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). *J Exp Med.* 1987; 166(4):1084-1097. (Clone-specific)
Luster AD, Unkeless JC, Ravetch JV. Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Nature.* 1985; 315(6021):672-676. (Clone-specific)
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: IC/FCM Block)