Technical Data Sheet

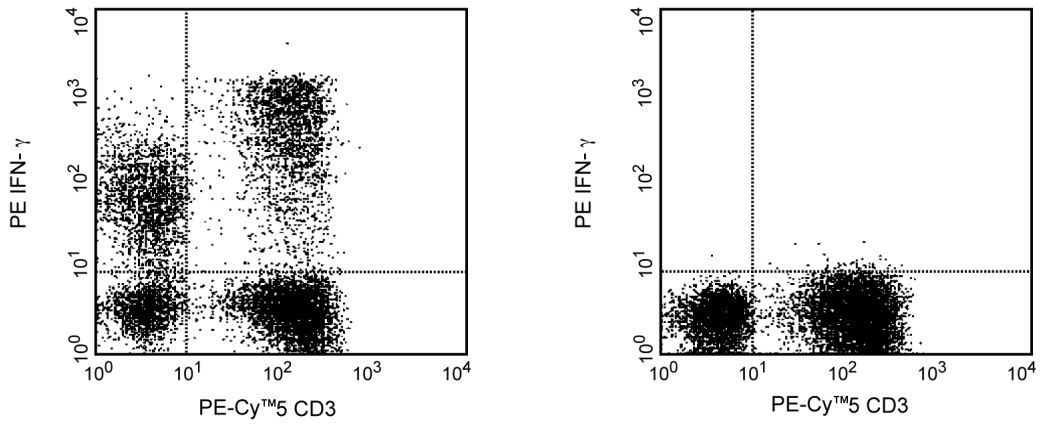
PE Mouse Anti-Human IFN-γ

Product Information

Material Number:	**562016**
Alternate Name:	IFNG; Interferon-gamma; Interferon-γ; Type II interferon; MAF
Size:	25 Tests
Vol. per Test:	20 µl
Clone:	B27
Immunogen:	Human IFN-γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The B27 monoclonal antibody specifically binds to human interferon-γ (IFN-γ), a 14-18 kDa glycoprotein containing 143 amino acid residues. IFN-γ is a potent multifunctional cytokine produced by several activated cell types including NK, NKT, CD4+TCRαβ+, CD8+TCRαβ+, and TCRγδ+ T cells. IFN-γ exerts its biological effects through specific binding to the high-affinity IFN-γ receptor complex comprised of IFN-γRα (CD119) and IFN-γRβ subunits. In addition to its antiviral effects, IFN-γ upregulates a number of lymphoid cell functions including the antimicrobial and anti-tumor responses of macrophages, NK cells, and neutrophils. In addition, IFN-γ influences the regulation of proliferation, differentiation, and effector responses of B cell and T cell subsets. These influences can involve IFN-γ's capacity to boost MHC class I and II expression by antigen-presenting cells as well as direct effects on B cells and T cells themselves. B27 is a neutralizing antibody. The use of B27 antibody for epitope mapping of human IFN-γ has been described. The B27 antibody has been reported not to bind to denatured IFN-γ.



Expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 h with PMA (50 ng/ml; Sigma, Cat. #P-8139) and calcium ionophore A23187 (250 ng/ml; Sigma, Cat. #C-9275) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554715). The PBMC were stained with PE-Cy™5 Mouse Anti-Human CD3 (Cat. 555334), fixed, permeabilized, and subsequently stained with 20 µl of PE Mouse Anti-Human IFN-γ (Cat. No. 559327/559327/554701; left panel). To demonstrate specificity of staining, binding by the PE-B27 antibody was blocked by preincubation of fixed/permeabilized cells with Purified Mouse Anti-Human IFN-γ (5 µg; Cat. No. 554699; right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on autofluorescence controls 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.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated B27 antibody is useful for flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations (see image). This 25 Test Size formulation of the PE-conjugated B27 antibody has been pre-titrated to assure effective intracellular detection of human IFN-γ using 20 µl per 1 x 10^6 cells. For specific methodology, please visit the protcols section of our website, http://www.bdbiosciences.com/support/resources/, under "Cytokines (Intracellular Staining)" and "Intracellular Flow".

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 (Cat. No. 559320). A useful control for demonstrating specificity of staining is the following: pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled B27 antibody (Cat. No. 554699) prior to staining. The intracellular cytokine staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe.

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. 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 x 10^6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X Perm/Wash™ Buffer.
2. Incubate the cell suspension for 15 minutes (4°C, in the dark).
3. Wash twice in 100 µl of 1X Perm/Wash™ Buffer.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
554699	Purified Mouse Anti-Human IFN-γ	0.1 mg	B27
559320	PE Mouse IgG1, κ Isotype Control	100 Tests	MOPC-21
555061	HiCK-1 Human Cytokine Positive Control Cells	1 mL	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
559327	PE Mouse Anti-Human IFN-γ	100 Tests	B27
554657	Stain Buffer (BSA)	500 mL	(none)
554701	PE Mouse Anti-Human IFN-γ	0.1 mg	B27
555334	PE-Cy™5 Mouse Anti-Human CD3	100 Tests	UCHT1

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. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific)

Favre C, Wijdenes J, Cabrillat H, Djossou O, Banchereau J, de Vries JE. Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. *Mol Immunol.* 1989; 26(1):17-25. (Clone-specific: Immunoprecipitation, Neutralization)

Fonteneau JF, Le Drean E, Le Guiner S, Gervois N, Diez E, Jotereau F. Heterogeneity of biologic responses of melanoma-specific CTL. *J Immunol.* 1997; 159(6):2831-2839. (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)

Rotteveel FT, Kokkelink I, van Lier RA, et al. Clonal analysis of functionally distinct human CD4+ T cell subsets. *J Exp Med.* 1988; 168(5):1659-1673. (Biology)