Technical Data Sheet

BV786 Mouse Anti-Human IFN-γ

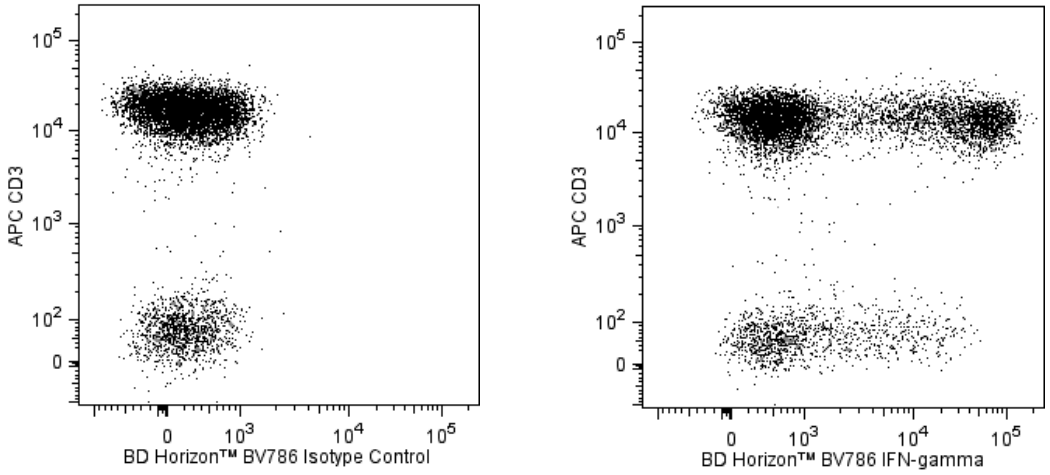
Product Information

Material Number:	**563731**
Alternate Name:	IFNG; Interferon-gamma; IFG; IFI; Type II interferon
Size:	50 tests
Vol. per Test:	5 µl
Clone:	4S.B3
Immunogen:	Partially purified human IFN-γ from supernatants of human PBMC stimulated with Staphylococcus aureus
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 4S.B3 monoclonal antibody specifically binds to interferon-γ (IFN-γ). The immunogen used to generate this hybridoma was partially purified human IFN-γ obtained from supernatants of human PBMC stimulated with *Staphylococcus aureus*. Interferon-γ (IFN-γ) is a potent multifunctional cytokine that is 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 antitumor responses of macrophages, NK cells, and neutrophils. In addition, IFN-γ can exert strong regulatory influences on the 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 to direct effects on B cells and T cells themselves. Human IFN-γ is a 14-18 kDa glycoprotein containing 143 amino acid residues.

The antibody was conjugated to BD Horizon™ BV786 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 786-nm. BD Horizon™ BV786 can be excited by the violet laser and detected in a filter used to detect Cy™7-like dyes (eg, 780/60-nm filter).



Two-color flow cytometric analysis of IFN-γ expression in stimulated human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were stimulated for 6 hours with Phorbol 12-Myristate 13-Acetate (Sigma P-8139; 50 ng/ml final concentration) and Ionomycin (Sigma I-0634; 1 µg/ml final concentration) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (554722). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Mouse Anti-Human CD3 antibody (Cat.No. 555335/561810/561811) and either BD Horizon™ BV786 Mouse IgG1 κ Isotype Control (Cat. No. 563330; Left Panel) or BD Horizon™ BV786 Mouse Anti-Human IFN-γ antibody (Cat. No. 563731; Right Panel). Two-color flow cytometric dot plots show the correlated expression patterns of IFN-γ (or Ig Isotype control staining) versus CD3 for gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.*

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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 BD Horizon™ BV786 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV786 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563330	BV786 Mouse IgG1, k Isotype Control	50 µg	X40
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555335	APC Mouse Anti-Human CD3	100 tests	UCHT1
561810	APC Mouse Anti-Human CD3	25 tests	UCHT1
561811	APC Mouse Anti-Human CD3	500 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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Cy is a trademark of Amersham Biosciences Limited.
6. Brilliant Violet™ 421 is a trademark of Sirigen.
7. Brilliant Violet™ 786 is a trademark of Sirigen.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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. (Clone-specific: Flow cytometry)

Meager A. Characterization of interferons and immunoassays. In: Clemens MJ, Morris AG, Gearing AJH, ed. *Lymphokines and Interferons. A Practical Approach.* Oxford: IRL Press Ltd; 1987:105-127. (Methodology)

Meager A, Parti S, Barwick S, Spragg J, O'Hagan K. Detection of hybridomas secreting monoclonal antibodies to human gamma interferon using a rapid screening technique and specificity of certain monoclonal antibodies to gamma interferon. *J Interferon Res.* 1984; 4(4):619-625. (Immunogen: Immunoprecipitation, Radioimmunoassay)

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, Kuenen B, Meager A, Miedema F, Lucas CJ. Clonal analysis of functionally distinct human CD4+ T cell subsets. *J Exp Med.* 1988; 168(5):1659-1673. (Clone-specific: ELISA)

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