Technical Data Sheet

BB700 Mouse Anti-Human IFN-γ

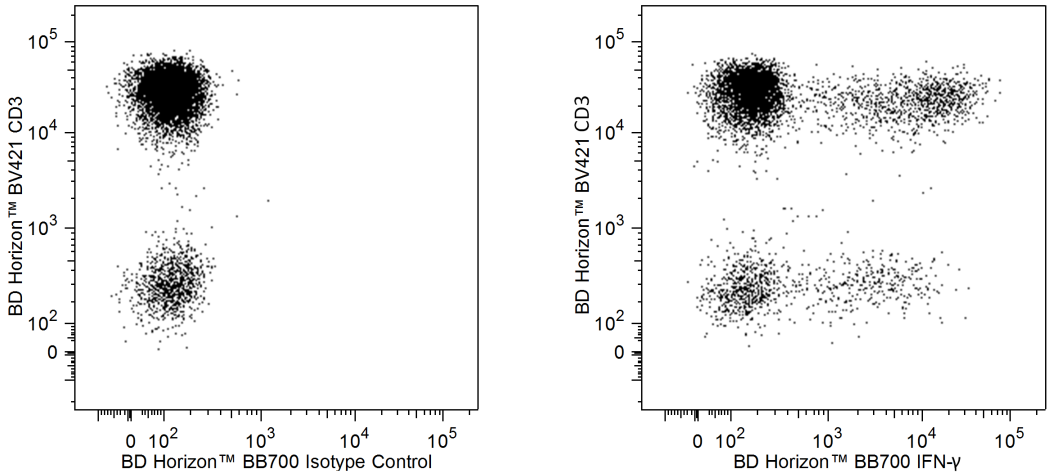
Product Information

Material Number:	**566394**
Alternate Name:	IFNG; Interferon-gamma; Interferon-γ; Type II interferon; MAF
Size:	100 Tests
Vol. per Test:	5 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 ≤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.

The antibody was conjugated to BD Horizon BB700, which is part of the BD Horizon Brilliant™ Blue family of dyes. It is a polymer-based tandem dye developed exclusively by BD Biosciences. With an excitation max of 485 nm and an emission max of 693 nm, BD Horizon BB700 can be excited by the 488 nm laser and detected in a standard PerCP-Cy™5.5 set (eg, 695/40-nm filter). This dye provides a much brighter alternative to PerCP-Cy5.5 with less cross laser excitation off the 405 nm and 355 nm lasers.



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 with BD Cytofix™ Fixation Buffer (Cat. No. 554655). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with BD Horizon™ BV421 Mouse Anti-Human CD3 antibody (Cat. No. 562426/562427) and either BD Horizon™ BB700 Mouse IgG1, κ Isotype Control (Cat. No. 566404; Left Plot) or BD Horizon BB700 Mouse Anti-Human IFN-γ antibody (Cat. No. 566394/566395; Right Plot). Two-color flow cytometric dot plots showing the correlated expression of IFN-γ (or Ig Isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 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™ BB700 under optimum conditions, and unconjugated antibody and free BD Horizon BB700 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794 or 566349).

When setting up compensation, it is recommended to compare spillover values obtained from cells and BD™ CompBeads to ensure that beads will provide sufficiently accurate spillover values.

For optimal results, it is recommended to perform two washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescent staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566404	BB700 Mouse IgG1, κ Isotype Control	50 µg	X40
566395	BB700 Mouse Anti-Human IFN-γ	25 Tests	B27
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
554655	Fixation Buffer	100 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
562427	BV421 Mouse Anti-Human CD3	25 Tests	UCHT1
562426	BV421 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. BD Horizon Brilliant Blue 700 is covered by one or more of the following US patents: 8,455,613 and 8,575,303.
8. Cy is a trademark of GE Healthcare.
9. An isotype control should be used at the same concentration as the antibody of interest.

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: ELISA, Neutralization)

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: Flow cytometry, 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, IC/FCM Block)

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)