

Technical Data Sheet

BV605 Mouse Anti-Human IFN- γ

Product Information

Material Number:	562974
Alternate Name:	IFNG; Interferon-gamma; Interferon- γ ; Type II interferon; MAF
Size:	50 Tests
Vol. per Test:	5 μ l
Clone:	B27
Immunogen:	Human IFN- γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

Description

The B27 monoclonal antibody specifically binds to human interferon- γ (IFN- γ). IFN- γ is a potent multifunctional cytokine that is produced by several activated cell types including NK, NKT, CD4+TCR $\alpha\beta$ +, CD8+TCR $\alpha\beta$ +, and TCR $\gamma\delta$ + T cells. IFN- γ exerts its biological effects through specific binding to the high-affinity IFN- γ Receptor Complex comprised of IFN- γ R α (CD119) and IFN- γ R β 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. 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 .

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

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).

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™ BV605 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV605 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
562652	BV605 Mouse IgG1, κ Isotype Control	50 μ g	X40
554656	Stain Buffer (FBS)	500 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
561811	APC Mouse Anti-Human CD3	500 Tests	UCHT1
555335	APC Mouse Anti-Human CD3	100 Tests	UCHT1
561810	APC Mouse Anti-Human CD3	25 Tests	UCHT1
563794	Brilliant Stain Buffer	5 mL	(none)

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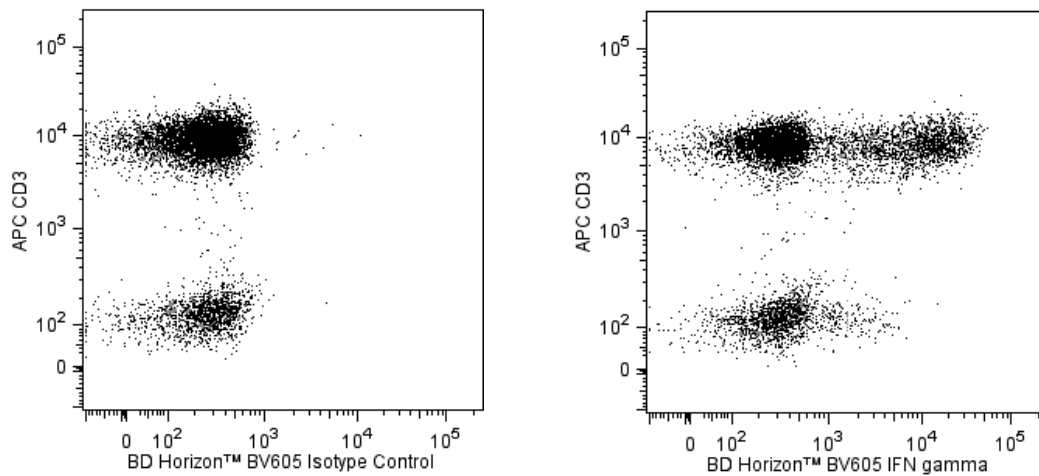
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Multicolor flow cytometric analysis of IFN- γ expressed in stimulated human peripheral blood mononuclear cells. HiCK-1 Human Cytokine Positive Control Cells (Cat. No. 555061) were permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723). The cells were then stained with either a BD Horizon™ BV605 Mouse IgG1, κ Isotype Control (Cat No. 562652, Left Panel) or with the BD Horizon™ BV605 Mouse Anti-Human IFN- γ antibody (Cat No. 562974, Right Panel) in conjunction with a APC Mouse Anti-Human CD3 antibody (Cat. No. 561811/555335/561810). Two-color flow cytometric dot plots showing the 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 cytometry was performed using a BD LSRFortessa™ Cell Analyzer System.

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- μ l experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon™ BV605 conjugate.
9. CF™ is a trademark of Biotium, Inc.

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, Immunoprecipitation)
 Favre C, Wijdenes J, Cabrilat 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)
 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)

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