

## Technical Data Sheet

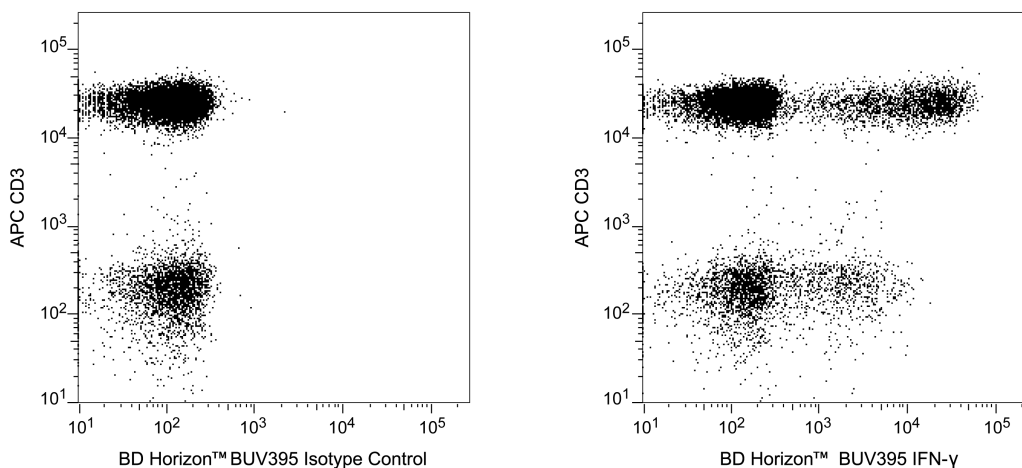
**BUV395 Mouse Anti-Human IFN-γ****Product Information**

<b>Material Number:</b>	<b>563563</b>
<b>Alternate Name:</b>	IFNG; Interferon-gamma; Interferon-γ; Type II interferon; MAF
<b>Size:</b>	50 tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	B27
<b>Immunogen:</b>	Human IFN-γ Recombinant Protein
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA and ≤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+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. 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™ BUV395 which has been exclusively developed by BD Biosciences as an optimal dye for use on a 355 nm laser equipped instrument. With an Ex Max at 348 nm and an Em Max at 395 nm, this dye has virtually no spillover into any other detector. BD Horizon™ BUV395 can be excited with a 355 nm laser and detected with a 379/28 filter.



**Two-color flow cytometric analysis of IFN-γ expression 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 APC Mouse Anti-Human CD3 antibody (Cat. No. 561811/555335/561810) and either BD Horizon™ BUV395 Mouse IgG1, κ Isotype Control (Cat. No. 563547, Left Panel) or BD Horizon™ BUV395 Mouse Anti-Human IFN-γ antibody (Cat. No. 563563, Right Panel). 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 cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

**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™ BUV395 under optimum conditions, and unconjugated antibody and free BD Horizon™ BUV395 were removed.

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## Application Notes

### Application

Intracellular staining (flow cytometry)

Routinely Tested

### Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563547	BUV395 Mouse IgG1, k Isotype Control	50 µg	X40
555061	HiCK-1 Human Cytokine Positive Control Cells	1.0 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

### Product Notices

1. An isotype control should be used at the same concentration as the antibody of interest.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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](http://www.bdbiosciences.com/colors).
5. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
6. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-µl experimental sample (a test).

### 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, 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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