Technical Data Sheet

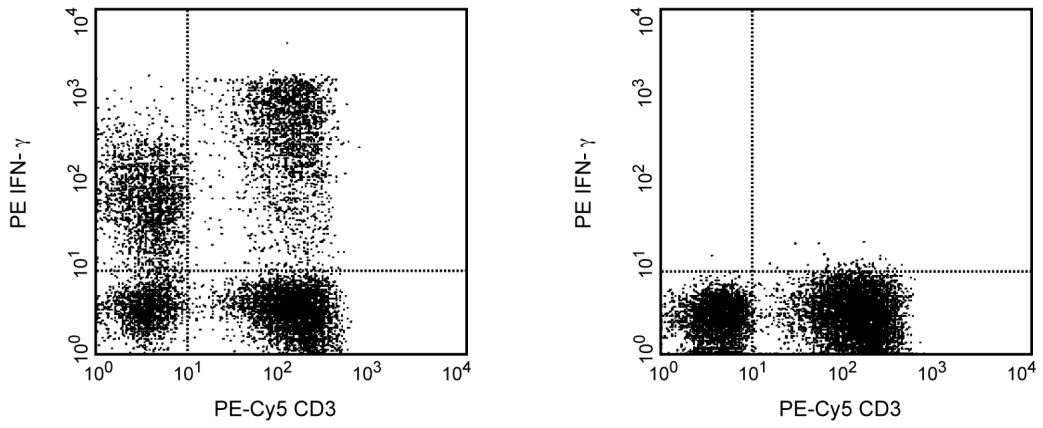
Purified Mouse Anti-Human IFN-γ

Product Information

Material Number:	**554699**
Alternate Name:	IFNG; Interferon-gamma; Interferon-γ; Type II interferon; MAF
Size:	0.1 mg
Concentration:	0.5 mg/ml
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.



Expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with PMA (50 ng/ml; Sigma, Cat. No. P-8139) and calcium ionophore A23187 (250 ng/ml; Sigma, Cat. No. C-9275) in the presence of GolgiStop™ (Cat. No. 554724). The PBMC were stained with PE-Cy™5 Mouse Anti-Human CD3 (Cat. No. 555334/561007), fixed and permeabilized with BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop) (Cat. No. 554715), and subsequently stained with 0.25 µg of PE Mouse Anti-Human IFN-γ (Cat. No. 554701/559327). To demonstrate staining specificity, PE Mouse Anti-Human IFN-γ antibody binding was blocked by preincubation of fixed/permeabilized cells with excess Purified Mouse Anti-Human IFN-γ (5 µg; Cat. No. 554699/550011; 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.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

ELISA	Routinely Tested
Neutralization	Tested During Development
Blocking	Tested During Development
Immunoprecipitation	Reported

Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified B27 antibody can be used as a blocking control to demonstrate specificity of IFN-γ staining by PE-B27 antibody or FITC-B27 antibody (Cat. No. 554701/554700). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1-10 µg of unlabeled B27 antibody (Cat. No. 554669) for 20 minutes at 4°C, prior to staining with PE-B27 antibody or FITC-B27 antibody (e.g., 0.1-0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Neutralization: The NA/LE™ B27 antibody is useful for neutralization of human IFN-γ bioactivity. This purified B27 antibody (Cat. No. 554698) is supplied in sodium azide free, sterile-filtered (0.22 µm pore) PBS, pH 7.2. Endotoxin level as determined by LAL assay is less than 0.01 ng/µg protein.

IP/WB: The B27 antibody has been reported to be useful for immunoprecipitation studies. The B27 antibody has been reported not to bind to denatured IFN-γ.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554701	PE Mouse Anti-Human IFN-γ	0.1 mg	B27
554698	Purified NA/LE Mouse Anti-Human IFN-γ	0.5 mg	B27
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
550011	Purified Mouse Anti-Human IFN-γ	0.25 mg	B27
555334	PE-Cy™5 Mouse Anti-Human CD3	100 Tests	UCHT1
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
561007	PE-Cy™5 Mouse Anti-Human CD3	25 Tests	UCHT1
559327	PE Mouse Anti-Human IFN-γ	100 Tests	B27
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
5. Cy is a trademark of GE Healthcare.
6. 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, Western blot)

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: 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)