Technical Data Sheet

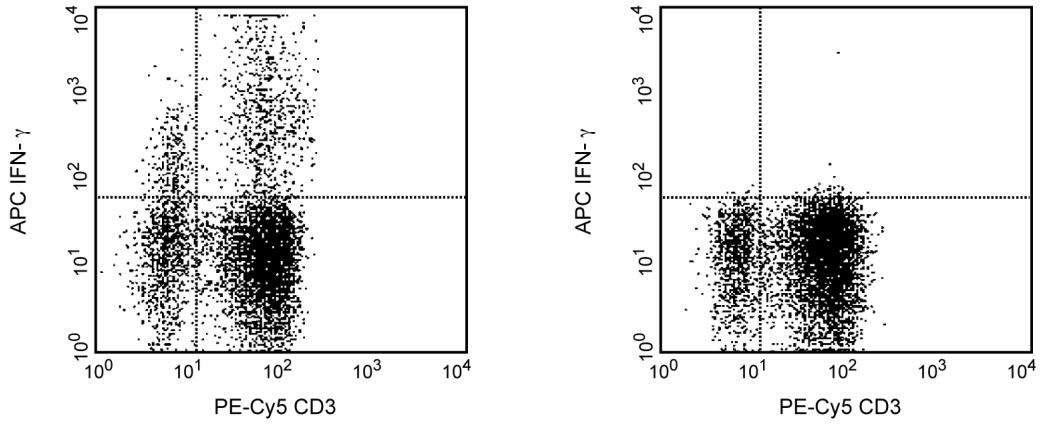
APC Mouse Anti-Human IFN-γ

Product Information

Material Number:	**562017**
Alternate Name:	IFNG; Interferon-gamma; Interferon-γ; Type II interferon; MAF
Size:	25 µg
Concentration:	0.2 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 protein stabilizer and ≤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 h 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 BD GolgiStop™ (2 µM; Cat. No. 554715). The PBMC were stained with PE-Cy™5 Mouse Anti-Human CD3 (Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of APC Mouse Anti-Human IFN-γ (Cat. No. 562017/554702, left panel). To demonstrate specificity of staining, binding by the APC-B27 antibody was blocked by preincubation of fixed/permeabilized cells with purified B27 antibody (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 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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The APC-conjugated B27 antibody (Cat. No. 554702/562017) is useful for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations (see image). For optimal immunofluorescent staining for flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit the protocols section under "Cytokines (Intracellular Staining)" or "Intracellular Flow" on our web site, www.bdbiosciences.com/us/s/resources.

A useful control for demonstrating specificity of staining is as follows: pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled B27 antibody (Cat. No. 554699/550011) prior to staining. The intracellular cytokine staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1κ isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is APC MOPC-21 (Cat. No. 554681).

Neutralization: The BD NA/LE™ B27 antibody (Cat. No. 554698) is useful for neutralization of human IFN-γ bioactivity. A suitable NA/LE mouse IgG1κ isotype control to match NA/LE B27 is the 107.3 antibody, (Cat. No. 554721/553447).

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. Please note that this application is not routinely tested.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
554699	Purified Mouse Anti-Human IFN-γ	0.1 mg	B27
554681	APC Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
555334	PE-Cy™5 Mouse Anti-Human CD3	100 Tests	UCHT1
554702	APC Mouse Anti-Human IFN-γ	0.1 mg	B27
550011	Purified Mouse Anti-Human IFN-γ	0.25 mg	B27
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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)

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)

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)