Technical Data Sheet

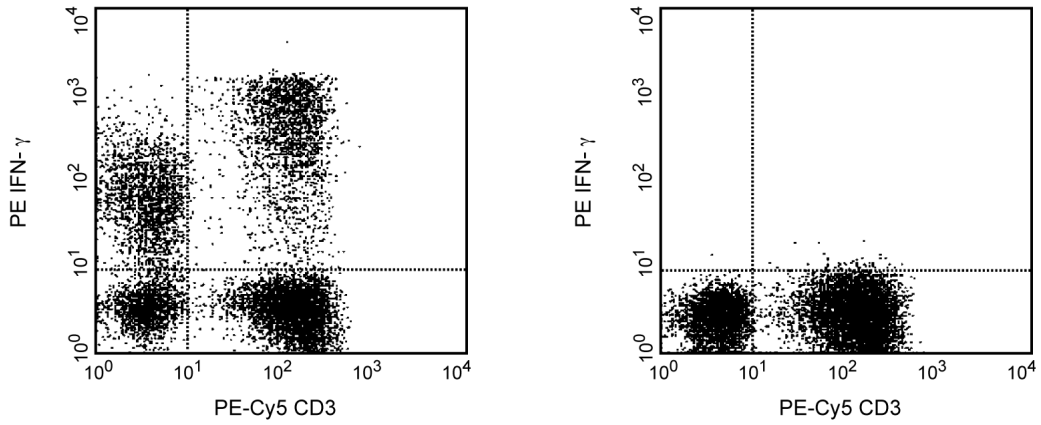
PE Mouse Anti-Human IFN-γ

Product Information

Material Number:	**554701**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	B27
Immunogen:	Human IFN-γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The B27 monoclonal antibody specifically binds to human interferon-γ (IFN-γ). This 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) and calcium ionophore A23187 (250 ng/ml; Sigma, Cat. #C-9275) in the presence of GolgiStop™ (2 µM; Cat. No. 554724). The PBMC were stained with PE-Cy5-anti-CD3 (PE-Cy5-UCHT1, Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of PE-mouse anti-human IFN-γ antibody (PE-B27, Cat. No. 554701), following Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, binding by the PE-B27 antibody was blocked by preincubation of fixed/permeabilized cells with unlabeled B27 antibody (5 µg; Cat. No. 554699; 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

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

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated B27 antibody 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

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immunofluorescent staining for flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

A useful control for demonstrating specificity of staining is the following: pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled B27 antibody (Cat. No. 554669) prior to staining. The intracellular cytokine staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is PE-MOPC-21 (Cat. No. 554680); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

Neutralization: The NA/LE™ B27 antibody (Cat. No. 554698) is useful for neutralization of human IFN-γ bioactivity. A suitable NA/LE™ mouse IgG1 isotype control to match the NA/LE B27 antibody is the 107.3 antibody, (Cat. No. 554721).

IP/WB: The PE-conjugated 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
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555061	HiCK-1 Human Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554699	Purified Mouse Anti-Human IFN-γ	0.1 mg	B27

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.

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)

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)