Technical Data Sheet

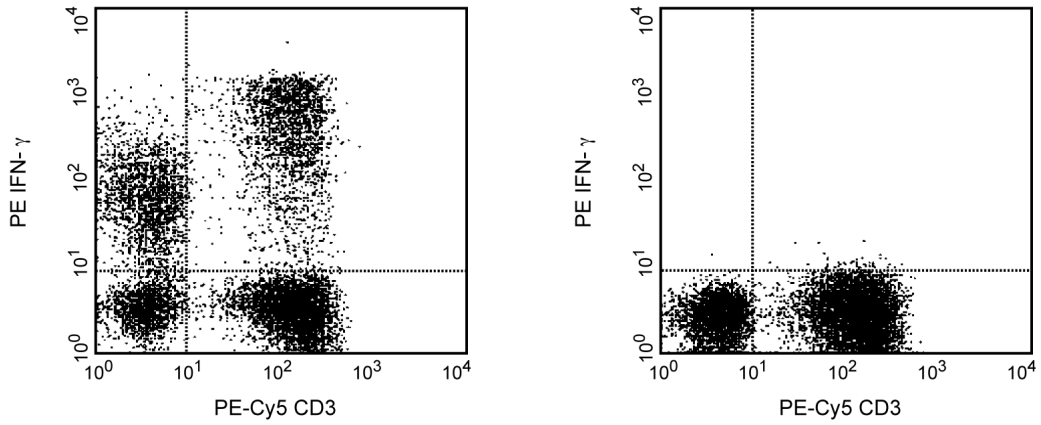
PE Mouse Anti-Human IFN-γ

Product Information

Material Number:	**559327**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	B27
Immunogen:	Human IFN-γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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, Cat. #P-8139) and calcium ionophore A23187 (250 ng/ml; Sigma, Cat. #C-9275) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were stained with PE-Cy5-anti-CD3 (PE-Cy5-UCHT1, Cat. 555334), fixed, permeabilized, and subsequently stained with 20 µl of PE-mouse anti-human IFN-γ antibody (PE-B27, Cat. No. 559327), by following the Usage section and 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). This 100 Test

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Size formulation of the PE-conjugated B27 antibody has been pre-titrated to assure effective intracellular detection of human IFN-γ using 20 µl per 1 x 10e6 cells. For specific methodology, please visit our website, http://www.bdbiosciences.com/pharmingen/protocols/ or refer to the chapter on intracellular staining in the Immune Function Handbook also posted on-line.

A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponinpermeabilized human cells is also available in a 100 Test Size formulation PE-MOPC-21 (Cat. No. 559320). 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. 554699) prior to staining. The intracellular cytokine staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe.

Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the USAGE section below.

USAGE

1. Resuspend 1 x 10^6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X Perm/Wash™ Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (4°C, in the dark).
3. Wash twice in 100 µl of 1X Perm/Wash™ Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
559320	PE Mouse IgG1, κ Isotype Control	100 tests	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)

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-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)