Technical Data Sheet

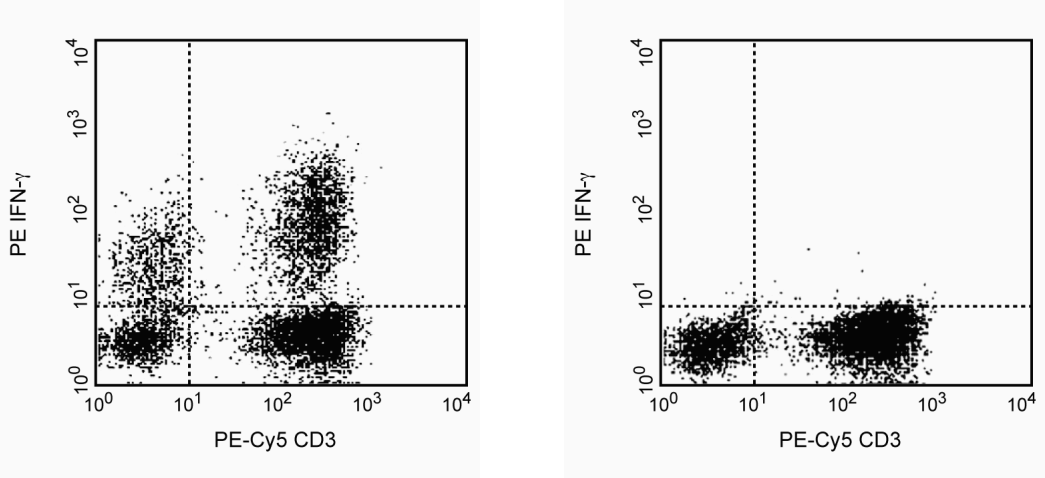
PE Mouse Anti-Human IFN-γ

Product Information

Material Number:	**554552**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	4S.B3
Immunogen:	Partially purified human IFN-γ from supernatants of human PBMC stimulated with Staphylococcus aureus
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 4S.B3 antibody reacts with human interferon-γ (IFN-γ). The immunogen used to generate this hybridoma was partially purified human IFN-γ obtained from supernatants of human PBMC stimulated with *Staphylococcus aureus*.



Expression of IFN- by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hr with PMA (50 ng/ml; Sigma) and calcium ionophore A23187 (500 ng/ml; Sigma) in the presence of 2 µM BD GolgiStop™ (Cat. No. 554724). The PBMC were stained with PE-Cy5-anti-CD3 (Pe-Cy5-UCHT1, Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.125 µg of PE-mouse anti-human IFN-γ antibody (PE-4S.B3) by using Pharmingen's staining protocol (left panel). The binding of PE-4S.B3 was blocked by preincubation of cells with unlabeled 4S.B3 antibody (Cat. No. 554549, 5 µg; right panel). The quadrant markers for the bivariate dot plot were set based on the autofluorescence controls and verified using the unlabeled antibody and ligand blocking controls.*

Preparation and Storage

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
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 for Flow Cytometric Analysis: The PE-4S.B3 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. For optimal immunofluorescent staining for flow cytometric analysis, the 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.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the PE-conjugated 4S.B3 antibody with ligand (e.g., rhIFN-γ, Cat. No. 554313) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled 4S.B3 antibody (Cat. No. 554549) prior to staining. The 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).

ELISA Detection: In its biotinylated form (Cat. No. 554550), the 4S.B3 antibody can be used as the detection antibody in a sandwich ELISA for measuring human IFN-γ protein levels in conjunction with purified NIB42 antibody (Cat. No. 551221) as the capture antibody and recombinant human IFN-γ (Cat. No. 554616) as the standard. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on ELISA in the Immune Function Handbook.

Note: This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. These ELISA reagents are not recommended for assaying serum or plasma samples. For measuring human IFN-γ in serum or plasma our humanFN-γ BD OptEIA set (Cat. No. 555142) or BD OptEIA kit (Cat. No. 550612) are specially formulated and recommended.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21

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

Meager A. Characterization of interferons and immunoassays. In: Clemens MJ, Morris AG, Gearing AJH, ed. *Lymphockies and Interferons. A Practical Approach.* Oxford: IRL Press Ltd; 1987:105-127. (Biology)

Meager A, Parti S, Barwick S, Spragg J, O'Hagan K. Detection of hybridomas secreting monoclonal antibodies to human gamma interferon using a rapid screening technique and specificity of certain monoclonal antibodies to gamma interferon. *J Interferon Res.* 1984; 4(4):619-625. (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)