Technical Data Sheet

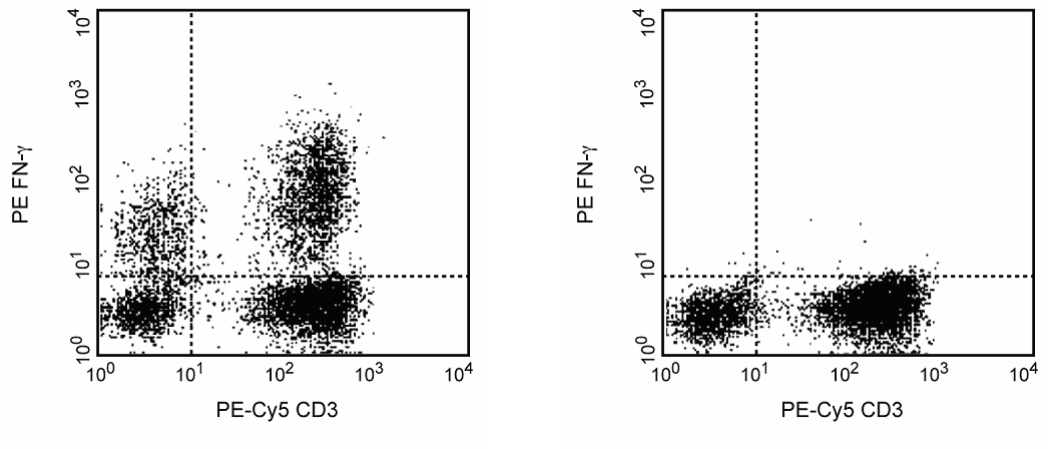
Purified Mouse Anti-Human IFN-γ

Product Information

Material Number:	**554549**
Size:	0.1 mg
Concentration:	0.5 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*. This is a neutralizing antibody.



Expression of IFN-γ 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, Cat. No. 554552) 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 (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 monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Intracellular block/flow cytometry	Routinely Tested
Western blot	Reported
ELISA Detection	Reported

Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified 4S.B3 antibody (Cat. No. 554549) can be used as a blocking control to demonstrate specificity of IFN-γ staining by directly conjugated clone 4S.B3. To perform this control, the fixed/permeabilized cells (~1 million) can be

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incubated with 1-10 µg of purified 4S.B3 antibody (Cat. No. 554549) for 20 minutes at 4°C, prior to staining with the directly conjugated antibody (e.g., 0.1 -0.5 µg mAb/1 million cells) (right panel). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. 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.

ELISA Detection: The biotinylated 4S.B3 antibody (Cat. No. 554550) is useful as a detection antibody in a sandwich ELISA for measuring human IFN-γ protein levels. Biotinylated 4S.B3 antibody can be paired with the purified NIB42 antibody (Cat. No. 551221) as the capture antibody, with recombinant human IFN-γ (Cat. No. 554616) as the standard. For testing human IFN-γ complex in biological fluids like serum or plasma, our human IFN-γ specific OptEIA™ sandwich ELISA set (Cat.No. 555142I) and OptEIA™ sandwich ELISA kit (Cat. No. 550612) are recommended.

Western Blot: The 4S.B3 antibody has been found useful for Western blotting.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555334	PE-Cy™5 Mouse Anti-Human CD3	100 tests	UCHT1
555332	FITC Mouse Anti-Human CD3	100 tests	UCHT1
554550	Biotin Mouse Anti-Human IFN-γ	0.5 mg	4S.B3
551221	Purified Mouse Anti-Human IFN-γ	1.0 mg	NIB42
554616	Recombinant Human IFN-γ	25 µg	(none)
550612	Human IFN-γ ELISA Kit II	2 plates	(none)
555142	Human IFN-γ ELISA Set	20 tests	(none)

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. 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)