Technical Data Sheet

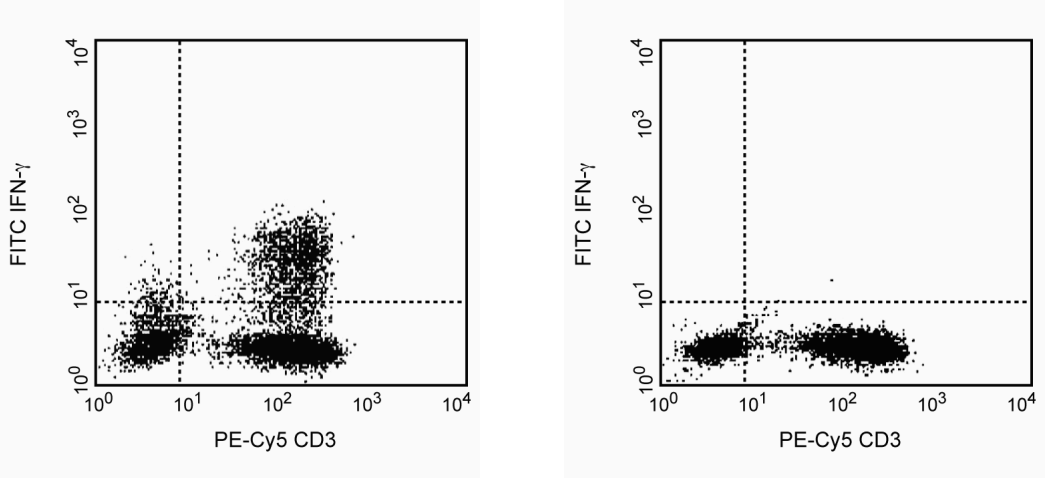
FITC Mouse Anti-Human IFN-γ

Product Information

Material Number:	**561057**
Size:	25 µg
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*.



Expression of IFN-γ by stimulated human periperal blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with PMA (50 ng/ml final concentration; Sigma) and calcium ionophore A23187 (500 ng/ml final concentration; Sigma) 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.No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of FITC mouse anti-human IFN-γ antibody (FITC 4S.B3, Cat. No.555334) (left panel). The binding of FITC 4S.B3 was blocked by preincubation of cells with unlabeled 4S.B3 antibody (5 µg; Cat. No. 554549; 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

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 with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: The FITC 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, this antibody should be titrated (≤ 0.5 µg mAb/million cells). A useful control for demonstrating specificity of staining is 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

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permeabilized human cells is FITC MOPC-21 (Cat. No. 554679); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554549	Purified Mouse Anti-Human IFN-γ	10 µg	4S.B3
554679	FITC Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555334	PE-Cy™5 Mouse Anti-Human CD3	100 tests	UCHT1
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554617	Recombinant Human IFN-γ	50 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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: Flow cytometry)