Technical Data Sheet

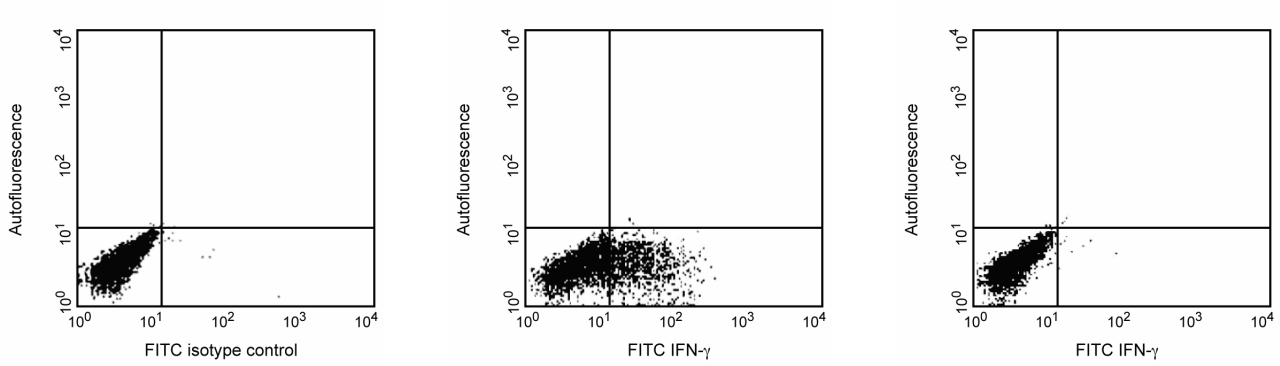
FITC Mouse Anti-Rat IFN-γ

Product Information

Material Number:	**559498**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	DB-1
Immunogen:	Recombinant Rat IFN-γ
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The DB-1 antibody reacts with rat interferon-γ (IFN-γ). The immunogen used to generate the DB-1 hybridoma was recombinant rat IFN-γ expressed in COS cells. This is a neutralizing antibody.



Expression of IFN-γ by stimulated LOU rat lymphoid cells. *Lymphoid cells from LOU rat were stimulated for 2 days with plate bound purified NA/LE™ mouse anti-rat CD3 (10 µg/ml; Cat. No. 554829), purified NA/LE™ mouse anti-rat CD28 (2 µg/ml; Cat. No.554993), recombinant rat IL-2 (10 ng/ml; Cat. No.555106) and recombinant rat IL-4 (50 ng/ml; Cat. No. 555107). The cells were then incubated for 3 days with recombinant rat IL-2 and IL-4. Following the 3 day incubation, the lymphoid cells were restimulated for 4 hours with PMA (5 ng/ml final concentration, Sigma, Cat. No.P-8139) and ionomycin (500 ng/ml final concentration, Sigma, Cat. No.I-0634) in the presence of GolgiPlug™ (1 µl/ml, Cat. No.555029). The activated cells were harvested, fixed, permeabilized, and subsequently stained with 20 µl of FITC- conjugated mouse anti-rat IFN- antibody (FITC-DB-1, Cat. No. 559498) by using Pharmingen' s staining protocol (see Center panel). To demonstrate specificity of staining, the binding by the FITC-DB-1 antibody was blocked by pre-incubation of the fixed/permeabilized cells with unlabeled DB-1 antibody (5 µg; Right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the staining profile using FITC-MOPC-21 isotype control antibody (Cat. No. 554679, see Left panel) and verified using the unlabeled antibody blocking specificity control.*

Preparation and Storage

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.
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 DB-1 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. This 100 Test Size formulation of the FITC-conjugated DB-1 antibody has been pre-titrated to assure effective intracellular detection of rat IFN-γ using 20 µl/1 x 10^6 cells in a final volume of 100 µl. 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 to pre-block the fixed/permeabilized cells with unlabeled DB-1 antibody prior to staining. The intracellular staining technique and the use of blocking controls have been 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/saponinpermeabilized rat cells is also available: FITC-MOPC-21 (Cat. No. 554679).

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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 permeabiliization agent saponin and is useful for this purpose as described below.

Resuspend one million fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X Perm/Wash™ Buffer (Cat. No. 554723). Incubate the cell suspension for 15 minutes (at RT or 4°C). Wash twice in 100 µl of 1X Perm/Wash™ Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554679	FITC Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554723	Perm/Wash Buffer	100 ml	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.

References

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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)

Schmidt B, Stoll G, van der Meide P, Jung S, Hartung HP. Transient cellular expression of gamma-interferon in myelin-induced and T-cell line-mediated experimental autoimmune neuritis. 1992; 115(Pt 6):1633-1646. (Biology)

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