Technical Data Sheet

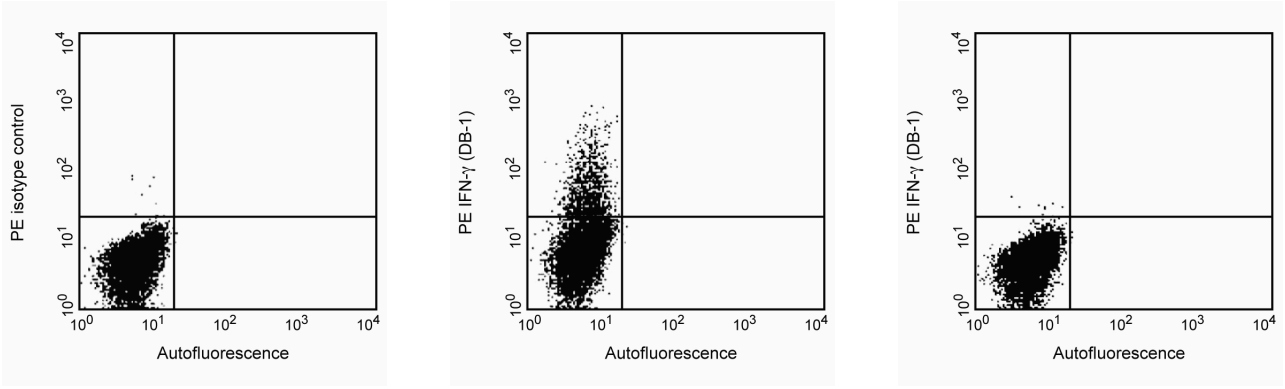
PE Mouse Anti-Rat IFN-γ

Product Information

Material Number:	**559499**
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 monoclonal antibody specifically binds to 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 spleen cells*. Splenocytes from a LOU rat were stimulated for 3 days with Concanavalin A (5 µg/ml final concentration; Sigma, Cat. #C-5275). The splenocytes were then restimulated for 4 hours with PMA (5 ng/ml final concentration, Sigma, Cat. # P-8139) and ionomycin (500 ng/ml final concentration, Sigma, Cat. #I-0634) in the presence of GolgiPlug™ (1 µl/ml, Cat. No. 555029). The splenocytes were harvested, fixed, permeabilized, and subsequently stained with 20 µl of PE-conjugated mouse anti-rat IFN-γ antibody (Cat. No. 559499) by using the BD Pharmingen staining protocol (Center Panel). To demonstrate specificity of staining, the binding by the PE-DB-1 antibody was blocked by pre-incubation of the fixed/permeabilized cells with unlabeled DB-1 antibody (10 µg; Right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the staining profile using PE-MOPC-21 isotype control antibody (Cat. No. 559320, see Left panel) and verified using the unlabeled antibody blocking specificity control.*

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular block/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 PE-conjugated DB-1 antibody has been pre-titrated to assure effective intracellular detection of rat IFN-γ using 20 µl/ million cells. The intracellular staining technique and the use of blocking controls have been described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com. A suitable mouse IgG1, κ isotype is also available in a 100 Test Size formulation PE-MOPC-21 (Cat. No. 559320).

Important Note: This pretitered 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

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

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

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 (at room temperature or 4°C).
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
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. An isotype control should be used at the same concentration as the antibody of interest.
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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Bakhiet M, Olsson T, Mhlanga J. Human and rodent interferon-gamma as a growth factor for Trypanosoma brucei. *Eur J Immunol.* 1996; 26(6):1359-1364. (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)

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)

van der Meide PH, Borman AH, Beljaars HG, Dubbeld MA, Botman CA, Schellekens H. Isolation and characterization of monoclonal antibodies directed to rat interferon-gamma. *Leuk Res.* 1989; 8(4):439-449. (Biology)

van der Meide PH, Borman TH, de Labie MC, et al. A sensitive two-site enzyme immunoassay for the detection of rat interferon-gamma in biological fluids. *J Interferon Res.* 1990; 10(2):183-189. (Biology)

van der Meide PH, Dubbeld M, Vijverberg K, Kos T, Schellekens H. The purification and characterization of rat gamma interferon by use of two monoclonal antibodies.. *J Gen Virol.* 1986; 67(Pt 6):1059-1071. (Biology)

van der Meide PH, Groenestein RJ, de Labie MC, Aten J, Weening JJ. Susceptibility to mercuric chloride-induced glomerulonephritis is age-dependent: study of the role of IFN-gamma. *Cell Immunol.* 1995; 162(1):131-137. (Biology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**