

Technical Data Sheet

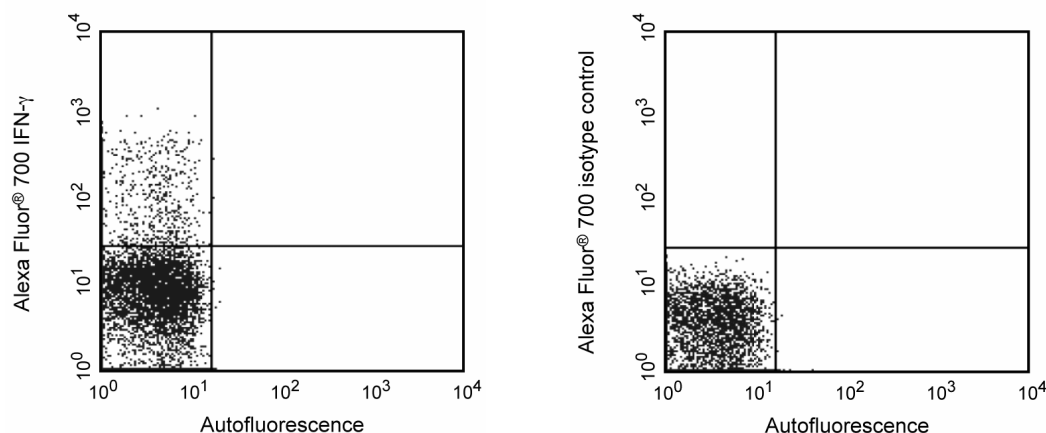
Alexa Fluor® 700 Mouse Anti-Human IFN- γ

Product Information

Material Number:	557995
Alternate Name:	IFNG; Interferon-gamma; Interferon- γ ; Type II interferon; MAF
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	B27
Immunogen:	Human IFN- γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and $\leq 0.09\%$ sodium azide.

Description

The B27 monoclonal antibody specifically binds to human interferon- γ (IFN- γ), a 14-18 kDa glycoprotein containing 143 amino acid residues. IFN- γ is a potent multifunctional cytokine produced by several activated cell types including NK, NKT, CD4+TCR $\alpha\beta$ +, CD8+TCR $\alpha\beta$ +, and TCR $\gamma\delta$ + T cells. IFN- γ exerts its biological effects through specific binding to the high-affinity IFN- γ receptor complex comprised of IFN- γ R α (CD119) and IFN- γ R β subunits. In addition to its antiviral effects, IFN- γ upregulates a number of lymphoid cell functions including the antimicrobial and anti-tumor responses of macrophages, NK cells, and neutrophils. In addition, IFN- γ influences the regulation of proliferation, differentiation, and effector responses of B cell and T cell subsets. These influences can involve IFN- γ 's capacity to boost MHC class I and II expression by antigen-presenting cells as well as direct effects on B cells and T cells themselves. B27 is a neutralizing antibody. The use of B27 antibody for epitope mapping of human IFN- γ has been described. The B27 antibody has been reported not to bind to denatured IFN- γ .



Expression of IFN- γ by stimulated human peripheral blood lymphocytes (PBMCs). Human PBMCs were stimulated with PMA and ionomycin in the presence of GolgiStop (Cat. No. 554715). The cells were harvested, fixed, permeabilized, and stained with either Alexa Fluor® 700 Mouse Anti-Human IFN- γ (Cat. No. 557995; left panel) or Alexa Fluor® 700 Mouse IgG1, κ Isotype Control (Cat. No. 557882; right panel). To demonstrate the specificity of staining, the binding of Alexa Fluor® 700 Mouse Anti-Human IFN- γ was blocked by preincubation of the fixed/permeabilized cells with an excess of Purified Mouse Anti-Human IFN- γ (10 μ g, Cat. No. 554699, data not shown) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype 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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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557995 Rev. 5



Recommended Assay Procedure:

The Alexa Fluor® 700-conjugated B27 antibody (Cat. No. 557995) 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 ($\leq 0.25 \mu\text{g mAb/million cells}$). For specific methodology, please visit the protocols section under "Intracellular Flow" or "Cytokines (Intracellular Staining)" on our web site, <http://www.bdbiosciences.com/us/s/resources>. A useful control for demonstrating specificity of staining is the unlabeled B27 antibody used to pre-block the fixed/permeabilized cells (Cat. No. 554699) prior to staining. A suitable mouse IgG1 isotype control for assessing the level of background staining is Alexa Fluor® 700-conjugated mAb MOPC-21 (Cat. No. 557882).

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
557882	Alexa Fluor® 700 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554699	Purified Mouse Anti-Human IFN- γ	0.1 mg	B27
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
555061	HiCK-1 Human Cytokine Positive Control Cells	1 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. Alexa Fluor® 700 has an adsorption maximum of $\sim 700\text{nm}$ and a peak fluorescence emission of $\sim 720\text{nm}$. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Favre C, Wijdenes J, Cabrillat H, Djossou O, Banchereau J, de Vries JE. Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. *Mol Immunol.* 1989; 26(1):17-25. (Clone-specific: Immunoprecipitation, Neutralization)

Fonteneau JF, Le Drean E, Le Guiner S, Gervois N, Diez E, Jotereau F. Heterogeneity of biologic responses of melanoma-specific CTL. *J Immunol.* 1997; 159(6):2831-2839. (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)

Rotteveel FT, Kokkelink I, van Lier RA, et al. Clonal analysis of functionally distinct human CD4+ T cell subsets. *J Exp Med.* 1988; 168(5):1659-1673. (Biology)