**BD Horizon™ Technical Data Sheet**

**BV421 Mouse Anti-Human IFN-γ**

**Product Information**

- **Material Number:** 564791
- **Alternate Name:** IFNG; Interferon-gamma; IFI; Type II interferon
- **Size:** 50 Tests
- **Vol. per Test:** 5 µl
- **Clone:** 4S.B3
- **Immunogen:** Human IFN-γ from supernatants of S. aureus-stimulated PBMC
- **Isotype:** Mouse (BALB/c) IgG1, κ
- **Reactivity:** QC Testing: Human
- **Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**

The 4S.B3 monoclonal antibody specifically binds to 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*. Interferon-γ (IFN-γ) is a potent multifunctional cytokine that is produced by several activated cell types including NK, NKT, CD4+ TCRαβ+, CD8+ TCRαβ+, and TCRγδ+ T cells. IFN-γ exerts its biological effects through specific binding to the high-affinity IFN-γ Receptor Complex comprised of IFN-γR1 (CD119) and IFN-γR2 subunits. In addition to its antiviral effects, IFN-γ upregulates a number of lymphoid cell functions including the antimicrobial and antitumor responses of macrophages, NK cells, and neutrophils. In addition, IFN-γ can exert strong regulatory influences on the 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 to direct effects on B cells and T cells themselves. Human IFN-γ is a 14-18 kDa glycoprotein containing 143 amino acid residues.

The antibody was conjugated to BD Horizon BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue conjugates.

**Two-color flow cytometric analysis of IFN-γ expression in stimulated human peripheral blood lymphocytes.**

Human peripheral blood mononuclear cells were stimulated for 6 hours with Phorbol 12-Myristate 13-Acetate (Sigma P-8139; 50 ng/ml final concentration) and Ionomycin (Sigma I-0634; 1 μg/ml final concentration) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (554722).

The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Mouse Anti-Human CD3 antibody (Cat. No. 555335/561810/561811) and either BD Horizon™ BV421 Mouse IgG1 κ Isotype Control (Cat. No. 562438; Left Panel) or BD Horizon BV421 Mouse Anti-Human IFN-γ antibody (Cat. No. 564791; Right Panel). Two-color flow cytometric contour plots showing the correlated expression patterns of IFN-γ (or Ig Isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.
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 BD Horizon™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

Application
- Intracellular staining (flow cytometry) Routinely Tested

Suggested Companion Products

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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. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References