Technical Data Sheet

# Purified Mouse Anti-Human IFN-γ

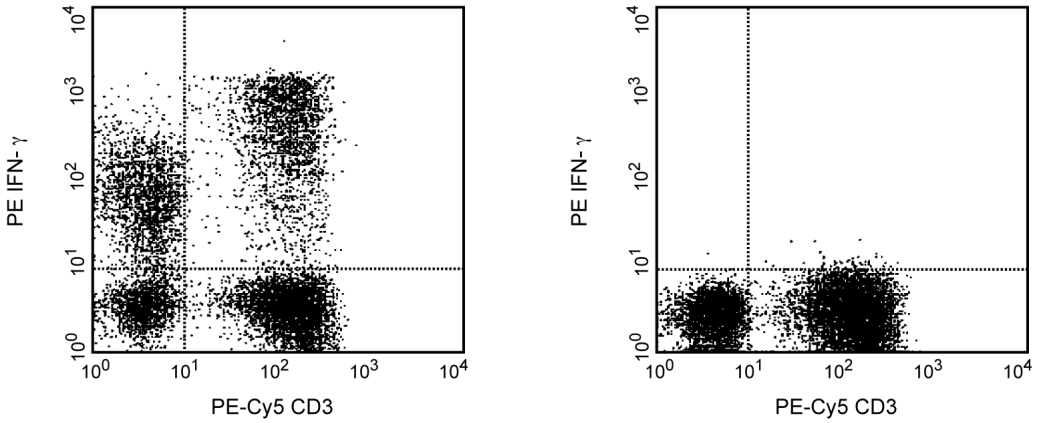
## Product Information

| | |
|---|---|
| **Material Number:** | **554699** |
| **Size:** | 0.1 mg |
| **Concentration:** | 0.5 mg/ml |
| **Clone:** | B27 |
| **Immunogen:** | Human E.coli-expressed IFN-γ Recombinant Protein |
| **Isotype:** | Mouse IgG1 κ |
| **Reactivity:** | QC Testing: Human |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

## Description

The B27 antibody reacts with human interferon-γ (IFN-γ). The immunogen used to generate the B27 hybridoma was *E.coli*-expressed recombinant human IFN-γ. This 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.

This antibody is routinely tested as a blocking control for intracellular staining. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.




***Expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC).*** *Human PBMC were stimulated for 6 hours with PMA (50 ng/ml; Sigma, Cat. No. P-8139) and calcium ionophore A23187 (250 ng/ml; Sigma, Cat. No. C-9275) in the presence of GolgiStop™ (2 µM; Sigma, Cat. No. M-5273). The PBMC were stained with PE-Cy5-anti-CD3 (Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of PE-mouse anti-human IFN-γ antibody (Cat. No. 554701), following the BD Pharmingen™ staining protocol (left panel). To demonstrate specificity of staining, binding by the PE-B27 antibody was blocked by preincubation of fixed/permeabilized cells with excess unlabeled B27 antibody (5 µg; Cat. No. 554699; right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on autofluorescence controls and verified using the unlabeled antibody blocking control.*

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

## Application Notes

**Application**

| | |
|---|---|
| IC/FCM Block | Routinely Tested |
| Immunoprecipitation | Reported |
| Neutralization | Reported |

BD Biosciences
bdbiosciences.com

| United States | Canada | Europe | Japan | Asia Pacific | Latin America/Caribbean |
|---|---|---|---|---|---|
| 877.232.8995 | 888.259.0187 | 32.53.720.550 | 0120.8555.90 | 65.6861.0633 | 55.11.5185.9995 |

For country-specific contact information, visit bdbiosciences.com/how_to_order/



BD
BD Biosciences

**Recommended Assay Procedure:**

**Blocking Control for Intracellular Staining:** The purified B27 antibody can be used as a blocking control to demonstrate specificity of IFN-γ staining by PE-B27 antibody or FITC-B27 antibody (Cat. No. 554701/554700). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1-10 µg of unlabeled B27 antibody (Cat. No. 554669) for 20 minutes at 4°C, prior to staining with PE-B27 antibody or FITC-B27 antibody (e.g., 0.1-0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

**Neutralization:** The NA/LE™ B27 antibody is useful for neutralization of human IFN-γ bioactivity. This purified B27 antibody (Cat. No. 554698) is supplied in sodium azide free, sterile-filtered (0.22 µm pore) PBS, pH 7.2. Endotoxin level as determined by LAL assay is less than 0.01 ng/µg protein. A suitable NA/LE™ mouse IgG1 isotype control to match the NA/LE™ B27 antibody is the 107.3 antibody, Cat. No. 554721.

**IP/WB:** The B27 antibody has been reported to be useful for immunoprecipitation studies. The B27 antibody has been reported not to bind to denatured IFN-γ.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 555061 | HiCK-1 Cytokine Positive Control Cells | 5x10^6 cells | (none) |
| 555334 | PE-Cy5 Mouse Anti-Human CD3 | 100 tests | UCHT1 |
| 554701 | PE Mouse Anti-Human IFN-γ | 0.1 mg | B27 |
| 554698 | NA/LE Mouse Anti-Human IFN-γ | 0.5 mg | B27 |

## Product Notices

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

## References


Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Clone-specific)

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, Western blot)

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: IC/FCM Block)