BD Horizon™ Technical Data Sheet

BUV737 Rat Anti-Mouse IFN-γ

Product Information

Material Number: 564693
Alternate Name: IFN-γ; IFN-g; IFN-gamma; Type II Interferon
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: XMG1.2
Immunogen: Mouse IFN-γ Recombinant Protein
Isotype: Rat IgG1, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The XMG1.2 monoclonal antibody specifically binds to mouse interferon-γ (IFN-γ) protein. IFN-γ is a pleiotropic cytokine, of approximately 15-17 kDa, involved in the regulation of inflammatory and immune responses. It plays an important role in activation, growth, and differentiation of T and B lymphocytes, macrophages, NK cells and other non-hematopoietic cell types. IFN-γ production is associated with the Th1 cell differentiation. The purified form of this antibody has been reported to be a neutralizing antibody.

The antibody was conjugated to BD Horizon BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome of BD Horizon BUV395 with an Ex Max of 348-nm and an acceptor dye with an Em Max at 737-nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 filter. Due to the excitation of the acceptor dye by other laser lines, there may be significant spillover into channels detecting Alexa Fluor® 700-like dyes (eg, 712/20-nm filter).

Due to spectral differences between labeled cells and beads, using BD™ CompBeads can result in incorrect spillover values when used with BD Horizon BUV737 reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different BUV737 reagents (e.g., CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.

Two-color flow cytometric analysis of IFN-γ expression by stimulated mouse splenocytes. Mouse splenic leucocytes were stimulated for 5 hours with Phorbol 12-Myristate 13-Acetate (PMA; Sigma P-8139; 50 ng/ml) and Ionomycin (Sigma I-0634; 1 µg/ml) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142) and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655).

The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Rat Anti-Mouse CD4 antibody (Cat. No. 553051/561091) and either BD Horizon™ BUV737 Rat IgG1, κ Isotype Control (Cat. No. 564690; Left Panel) or BD Horizon BUV737 Rat Anti-Mouse IFN-γ antibody (Cat. No. 564693; Right Panel) by using the BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric contour plots showing the correlated expression of IFN-γ (or Ig Isotype control staining) versus CD4 were derived from gated events with the forward and side light-scatter characteristics of intact stimulated leucocytes. 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™ BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes
Application

Intracellular staining (flow cytometry) Routinely Tested

Suggested Companion Products

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<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
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<tr>
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<td>564690</td>
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<td>Brilliant Stain Buffer</td>
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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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References