## Brilliant Violet 750<sup>™</sup> anti-human IFN-γ

Catalog # / Size:	3112750 / 100 tests 3112745 / 25 tests
Clone:	4S.B3
lsotype:	Mouse IgG1, к
Immunogen:	Partially purified, native human IFN- Y
<b>Reactivity:</b>	Human, Non-human primate, Other
Preparation:	The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 750™ under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Workshop Number:	HCDM listed
Concentration:	lot-specific



PMA/ionomycin-stimulated (4 hours) human peripheral blood lymphocytes were fixed, permeabilized and intracellularly stained with CD3 PE and IFN-γ (clone 4S.B3) Brilliant Violet 750<sup>™</sup> (left) or mouse IgG1, κ Brilliant Violet 750<sup>™</sup> isotype control (right).

## **Applications:**

Applications: Intracellular Flow Cytometry



Total cell lysates (15 µg protein) from serum-starved NIH/3T3 cells treated without (-) or with (+) 20% FBS for 30 minutes were resolved by 4-12% Bis-Tris gel electrophoresis, transferred to a PVDF membrane, and probed with 0.25 µg/mL (1:2000 dilution) of purified anti-RPS6 Phospho (Ser244) antibody (clone A18024A). Proteins were visualized by chemiluminescence detection using HRP goat antimouse IgG antibody at a 1:3000 dilution. Equal protein loading was confirmed using a purified anti-RPS6 antibody and Direct-Blot<sup>™</sup> HRP anti-GAPDH antibody used at a 1:25000 dilution

Recommended Usage: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5  $\mu$ L per million cells in 100  $\mu$ L staining volume or 5  $\mu$ L per 100  $\mu$ L of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

Brilliant Violet 750<sup>™</sup> excites at 405 nm and emits at 750 nm. The bandpass filter 780/60 nm is recommended for detection, although filter optimization may be required depending on other fluorophores used. Be sure to verify that your cytometer configuration and software setup are appropriate for detecting this channel. Refer to your instrument manual or manufacturer for support. Brilliant Violet 750<sup>™</sup> is a trademark of Sirigen Group Ltd.

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Application Notes: ELISA or ELISPOT Detection<sup>5</sup>: The biotinylated 4S.B3 antibody is useful as a detection antibody for a sandwich ELISA or ELISPOT assay, when used in conjunction with purified NIB42 antibody or purified MD-1 antibody as the capture antibody.

> **Flow Cytometry**<sup>3,4,6-8</sup>: The fluorochrome-labeled 4S.B3 antibody is useful for intracellular immunofluorescent staining and flow cytometric analysis to identify IFN-γ -producing cells within mixed cell populations.

## Additional reported applications (for the relevant formats)

**include:** neutralization<sup>1,2</sup>, Western blotting, immunohistochemical staining of paraformaldehyde-fixed, saponin-treated tissue sections, and immunocytochemistry. The 4S.B3 antibody can neutralize the bioactivity of natural or recombinant IFN- $\gamma$ . (lower). Lane M: molecular weight ladder.

Application	<ol> <li>Meager A, et al. 1984. J. Interferon Res. 4:619. (Neut)</li> <li>Meager A, 1987. Lymphokines and Interferons:A Practical Approach. IRL</li></ol>
References:	Press Ltd, Oxford, p. 105. (Neut) <li>Sester M, et al. 2002. J. Virol. 76:3748. (ICFC)</li> <li>Infante-Duarte C, et al. 2000 J. Immunol. 165:6107. (ICFC)</li> <li>Goodier M, et al. 2000. J. Immunol. 165:139. (ELISA)</li> <li>Chen H, et al. 2005. J. Immunol. 175:591. (ICFC)</li> <li>Smeltz RB, 2007. J. Immunol. 178:4786. (ICFC)</li> <li>Iwamoto S, et al. 2007. J. Immunol. 179:1449. (ICFC) PubMed</li> <li>Yoshino N, et al. 2000. Exp. Anim. (Tokyo) 49:97. (ICFC)</li>
Description:	Interferon- $\gamma$ is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Originally characterized based on anti- viral activities, IFN- $\gamma$ also exerts anti-proliferative, immunoregulatory, and proinflammatory activities. IFN- $\gamma$ can upregulate MHC class I and II antigen expression by antigen-presenting cells.
Antigen	<ol> <li>Fitzgerald K, et al. Eds. 2001. The Cytokine FactsBook. Academic Press,</li></ol>
References:	San Diego. <li>De Maeyer E, et al. 1992. Curr. Opin. Immunol. 4:321.</li> <li>Farrar M, et al. 1993. Annu. Rev. Immunol. 11:571.</li> <li>Gray P, et al. 1987. Lymphokines 13:151.</li>