Technical Data Sheet

PerCP-Cy™5.5 Rat Anti-Human GM-CSF

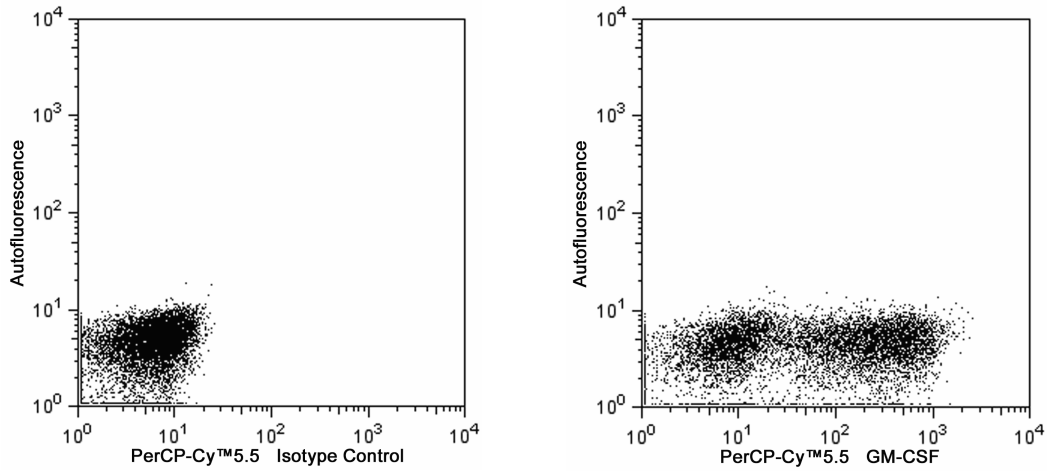
Product Information

Material Number:	**562258**
Alternate Name:	CSF2; Colony stimulating factor 2 (granulocyte-macrophage); CSF; GMCSF
Size:	50 tests
Vol. per Test:	5 µl
Clone:	BVD2-21C11
Immunogen:	Recombinant human GM-CSF
Isotype:	Rat (LEW) IgG2a
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BVD2-21C11 monoclonal antibody specifically binds to human Granulocyte/Macrophage - Colony Stimulating Factor (GM-CSF). Human GM-CSF is encoded by the *CSF2* gene and is also known as Colony Stimulating Factor 2. GM-CSF is produced by activated T lymphocytes, macrophages, endothelial cells, fibroblasts, stromal cells and other cell types including B lymphocytes, mast cells, eosinophils, and osteoblasts. GM-CSF stimulates the survival, proliferation and/or differentiation of various cell types including neutrophils, eosinophils, macrophages, dendritic cells, megakaryocytes, erythroid cells, endothelial cells and their precursors. The immunogen used to generate the BVD2-21C11 hybridoma was recombinant human GM-CSF. The BVD2-21C11 antibody has been reported to crossreact with GM-CSF from the rhesus monkey. BVD2-21C11 is a neutralizing antibody.

The binding of conjugated BVD2-21C11 antibody has been shown to be blocked by preincubation with recombinant human GM-CSF (0.1 µg; Cat. No. 550068) and by preincubation of the fixed/permeabilized cells with unlabeled BVD2-21C11 antibody (Cat. No. 554503) prior to staining. Please view the PE Rat anti-Human GM-CSF (Cat. No. 554507) Technical Data Sheet for additional data.



Multicolor analysis of GM-CSF expressed by HiCK-2 cells. *HiCK-2 (Human intracellular CytoKine-2) Cytokine Positive Control Cells (Cat. No. 555062) were permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723). The cells were then stained with either PerCP-Cy™5.5 Rat IgG2a, κ Isotype Control (Cat No. 550765, Left Panel) or PerCP-Cy™5.5 Mouse anti-Human GM-CSF antibody (Cat No. 562258, Right Panel) by using BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric dot plots showing the expressed levels of GM-CSF (or Ig isotype control staining) versus cellular autofluorescence (measured in the FL2 channel) were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry 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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
550765	PerCP-Cy™5.5 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
554723	Perm/Wash Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
555062	HiCK-2 Human Cytokine Positive Control Cells	1.0 ml	(none)

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
7. 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.
8. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
9. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
11. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1995:6.20-6.21. (Clone-specific: ELISA)

Abrams JS, Gleich GI, Van Dyke RE, Silver JE. Eosinophil-active cytokines in human disease: development and use of monoclonal antibodies to IL-3, IL-5, GMCSF. In: Gleich GJ and Kay AB, ed. *Eosinophils in Allergy and Inflammation.* New York: Dekker; 1994:133-157. (Clone-specific: ELISA, Neutralization)

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: ELISA, Immunoprecipitation, Neutralization)

Bacchetta R, de Waal Malefijt R, Yssel H. Host-reactive CD4+ and CD8+ T cell clones isolated from a human chimera produce IL-5, IL-2, IFN-gamma and granulocyte/macrophage-colony-stimulating factor but not IL-4. *J Immunol.* 1990; 144(3):902-908. (Clone-specific: ELISA, Neutralization)

Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med.* 1991; 174(3):745-748. (Clone-specific: ELISA, Neutralization)

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