Technical Data Sheet

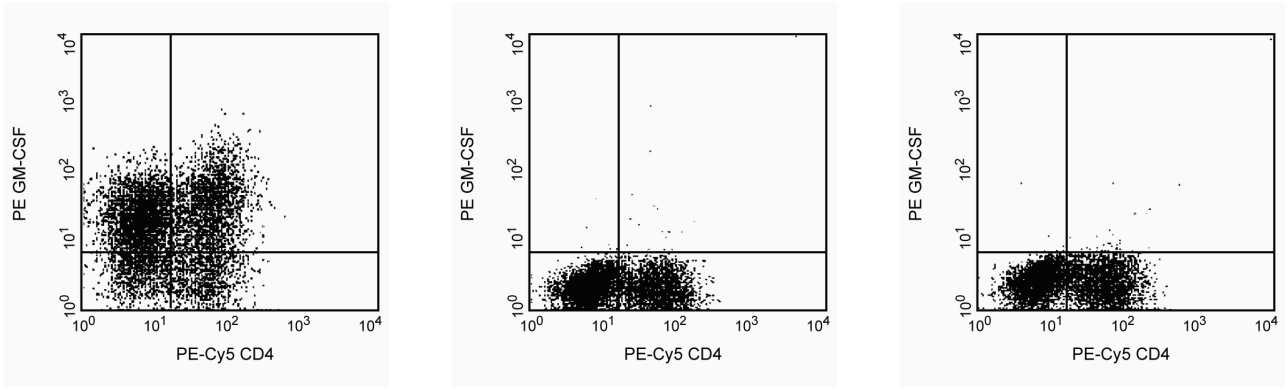
Purified Rat Anti-Human GM-CSF

Product Information

Material Number:	**554503**
Alternate Name:	CSF2; Colony stimulating factor 2 (granulocyte-macrophage); CSF; GMCSF
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	BVD2-21C11
Immunogen:	Recombinant human GM-CSF
Isotype:	Rat (LEW) IgG2a
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤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.



Expression of GM-CSF by stimulated human peripheral blood mononuclear cells (PBMC). *Ficoll™-separated human PBMC were stimulated with soluble anti-human CD3 antibody (1 µg/ml final concentration; UCHT1; Cat. No. 555329), recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 554603) and recombinant human IL-4 (10 ng/ml final concentration; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant human IL-4 for 3 days. Finally, the cells were harvested and stimulated for 6 hours with PMA (50 ng/ml final concentration; Sigma, Cat. #P- 8139) and calcium ionophore A23187 (250 ng/ml final concentration; Sigma, Cat. #C- 9275) in the presence of BD GolgiStop™ (2 µM final concentration; Cat. No. 554724). The cells were harvested, stained with PE-Cy™5 anti CD4 (Cat. No. 555348), fixed, permeabilized, and subsequently stained with 0.25 µg of PE Rat anti-Human GM-CSF antibody (PE-BVD2-21C11, Cat. No. 554503) by using BD Biosciences Pharmingen's staining protocol (Left panel). To demonstrate specificity of staining, the binding of the PE-BVD2-21C11 antibody was blocked by preincubation of the antibody conjugate with recombinant human GM-CSF (0.1 µg; Cat. No. 550068; Center panel) and by preincubation of the fixed/permeabilized cells with unlabeled BVD2-21C11 antibody (5 µg; Cat. No. 554503; Right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on the autofluorescence control and verified using the ligand-blocking and unlabeled antibody blocking controls.*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Intracellular block/flow cytometry	Tested During Development
Immunoprecipitation/Western blot	Reported

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Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified BVD2-21C11 antibody (Cat. No. 554503) can be used as a blocking control to demonstrate specificity of human GM-CSF staining by the PE-BVD2-21C11 (Cat. No. 554507). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1 - 10 µg of unlabeled BVD2-21C11 antibody (Cat. No. 554503) for 20 minutes at 4°C, prior to staining with PE-BVD2-21C11 antibody (eg, 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. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our website, www.bdbiosciences.com.

ELISA Detection: The biotinylated BVD2-21C11 antibody, Cat. No. 554505, is useful as a detection antibody for a sandwich ELISA for measuring human GM-CSF protein levels. Biotinylated BVD2-21C11 antibody can be paired with the purified BVD2-23B6 antibody (Cat. No. 554502) as the capture antibody, with recombinant human GM-CSF (Cat. No. 550068) as the standard. For specific methodology please visit the protocols sections or the chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com. This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. **Note**: For testing GM-CSF in serum or plasma, our BD OptEIA™ ELISA Set (Cat. No. 555126) is recommended.

IP: The purified BVD2-21C11 antibody has been reported to be useful for immunoprecipitation studies. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554507	PE Rat Anti-Human GM-CSF	0.1 mg	BVD2-21C11
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
550068	Recombinant Human GM-CSF	10 µg	(none)
555126	Human GM-CSF ELISA Set	20 plates	(none)
554502	Purified Rat Anti-Human GM-CSF	0.5 mg	BVD2-23B6
554505	Biotin Rat Anti-Human GM-CSF	0.5 mg	BVD2-21C11
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

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.
4. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
5. Cy is a trademark of Amersham Biosciences Limited.

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, 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)

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)

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)

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)

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