Technical Data Sheet

BV421 Mouse Anti-Human IL-8

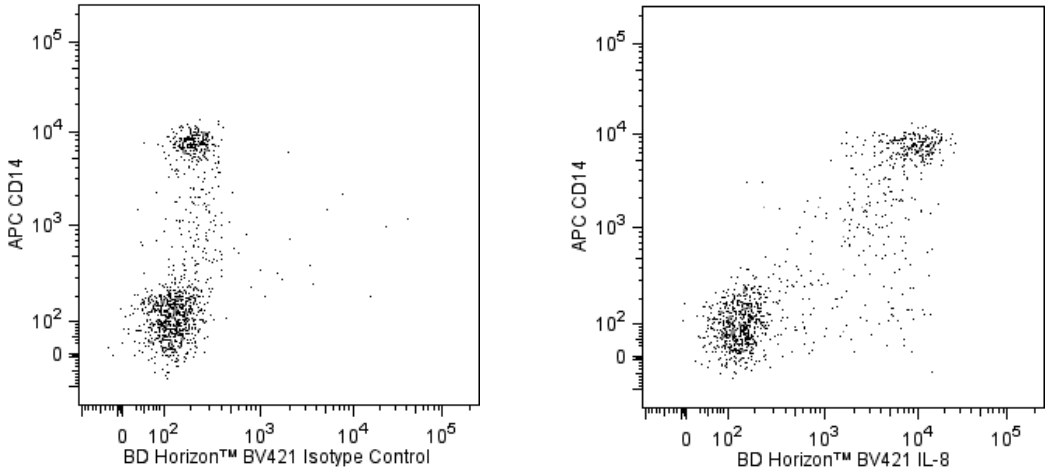
Product Information

Material Number:	**563310**
Alternate Name:	IL8; CXCL8; GCP-1; LYNAP; MDNCF; MONAP; NAP-1; emoctakin
Size:	50 tests
Vol. per Test:	5 µl
Clone:	G265-8
Immunogen:	Recombinant Human IL-8
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The G265-8 monoclonal antibody specifically binds to both the 72 and 77 amino acid isoforms of human Interleukin-8 (IL-8). IL-8 is secreted as an 8-9 kDa, non-glycosylated proinflammatory chemokine protein also known as chemokine (C-X-C motif) ligand 8 (CXCL8). IL-8 is synthesized as a 99 amino acid precursor that is proteolytically processed into several isoforms. The 72 amino acid isoform is produced by monocytes, macrophages, granulocytes, epithelial cells, and fibroblasts in response to pro-inflammatory stimuli including cytokines and microbial agents. It is also expressed by endothelial cells, fibroblasts, keratinocytes, lymphocytes, and a variety of tumor cells. In response to IL-4, IL-10 and TGFβ, the cellular production of IL-8 is inhibited. IL-8 is crucial for the activation and recruitment of neutrophils to inflammatory sites. IL-8 is also a chemoattractant for basophils and T-lymphocytes. IL-8 possesses angiogenic activity and can be associated with tumor angiogenesis and metastasis. The 77 amino acid IL-8 isoform is primarily produced by endothelial cells. This larger isoform is reportedly a less potent neutrophil activator than the 72 amino acid isoform. IL-8 binds to and signals through two G-protein-coupled receptors, IL-8RA (CXCR1/CD181) and IL-8RB (CXCR2/CD182).

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.



Two-color flow cytometric analysis of IL-8 expression in stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were primed for 2 hr with Recombinant Human IFN-γ (10 ng/ml; Cat. No. 554617/554616) and stimulated with lipopolysaccharide (1.0 µg/ml; Sigma, Cat. No. L-8274; 6 hr) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing monensin) (Cat. No. 554724). The PBMC were then stained with APC Mouse Anti-Human CD14 antibody (Cat. No. 555399/561708/561383). After washing with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), the cells were fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (Cat. No. 554722). The cells were then stained with either BD Horizon™ BV421 Mouse IgG2b, κ Isotype Control (Cat. No. 562748; Left Panel) or BD Horizon™ BV421 Anti-Human IL-8 antibody (Cat. No. 563310; Right Panel) using BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric dot plots show the correlated expression patterns of IL-8 (or Ig Isotype control staining) versus CD14 for gated events with the forward and side light-scatter characteristics of intact stimulated PBMC. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
562748	BV421 Mouse IgG2b, κ Isotype Control	50 µg	27-35
554617	Recombinant Human IFN-γ	50 µg	(none)
554616	Recombinant Human IFN-γ	25 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555399	APC Mouse Anti-Human CD14	100 tests	M5E2
561708	APC Mouse Anti-Human CD14	25 tests	M5E2
561383	APC Mouse Anti-Human CD14	50 tests	M5E2
554722	Fixation and Permeabilization Solution	125 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Brilliant Violet™ 421 is a trademark of Sirigen.

References

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