

## Technical Data Sheet

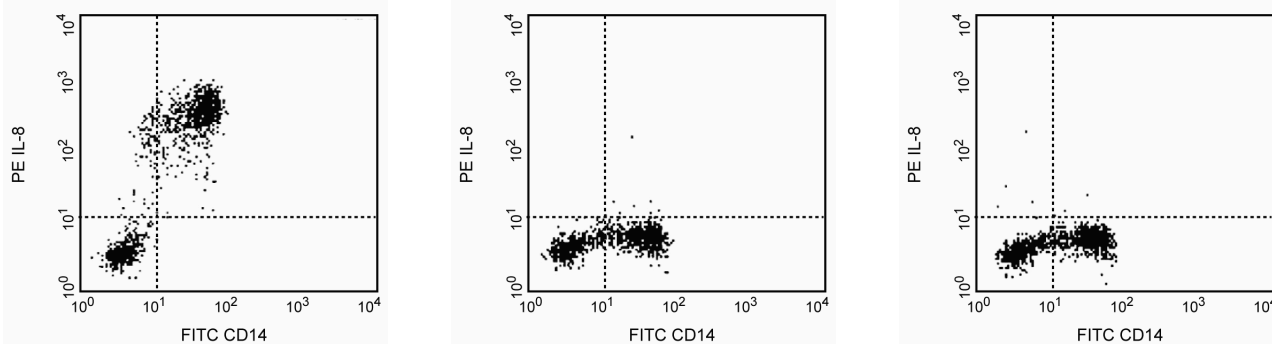
## PE Mouse Anti-Human IL-8

## Product Information

<b>Material Number:</b>	<b>554720</b>
<b>Alternate Name:</b>	IL8; CXCL8; GCP-1; LYNAP; MDNCF; MONAP; NAP-1; emoctakin
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	G265-8
<b>Immunogen:</b>	Recombinant Human IL-8
<b>Isotype:</b>	Mouse IgG2b
<b>Reactivity:</b>	QC Testing: Human
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤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).



**Expression of IL-8 by stimulated CD14<sup>+</sup> human monocytes.** Human PBMC were stimulated for 6 hours with LPS (10 ng/ml final concentration) in the presence of 2 μM GolgiStop™ (Cat. No. 554724). The PBMC were harvested, stained with FITC Mouse Anti-Human CD14 (Cat. No. 555397), fixed, permeabilized, and subsequently stained with 0.25 μg of PE Mouse Anti-Human IL-8 (Cat. No. 554720) following Pharmingen's staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, the binding of PE Mouse Anti-Human IL-8 was blocked by the preincubation of the conjugated antibody with recombinant human IL-8 (0.25 μg, Cat. No. 554609; center panel), and by preincubation of the fixed/permeabilized cells with Purified Mouse Anti-Human IL-8 (2.5 μg, Cat. No. 554717/550419; right panel) prior to staining with the PE Mouse Anti-Human IL-8. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (center) and unlabelled antibody (right) blocking specificity controls.

## 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

## Application Notes

## Application

Intracellular staining (flow cytometry)

Routinely Tested

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### Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The PE Mouse Anti-Human IL-8 (Cat. No. 554720) can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate IL-8 producing cells within mixed cell populations (see figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated ( $\leq 0.5 \mu\text{g}$  mAb/million cells). For specific methodology, please visit our protocols under "Intracellular Flow" at our website: <http://www.bdbiosciences.com/us/s/resources/>.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated G265-8 antibody with ligand (e.g., recombinant human IL-8; Cat. No. 554609) prior to staining, or 2) pre-block the fixed/ permeabilized cells with Purified Mouse Anti-Human IL-8 (Cat. No. 554717/550419) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. An appropriate PE-mouse IgG2b isotype control to use on fixed and permeabilized cells is PE Mouse IgG2b,  $\kappa$  Isotype Control (Cat. No. 555058).

Cytokine ICC: The G265-8 antibody is useful for immunocytochemical staining. Purified Mouse Anti-Human IL-8 (Cat. No. 550419/554717) is tested in the ICC application.

ELISA Detection: The Biotin Mouse Anti-Human IL-8 antibody (Catalog No. 554718) is useful as a detection antibody in a sandwich ELISA for measuring human IL-8 protein levels. Biotin Mouse Anti-Human IL-8 antibody can be paired with the Purified Mouse Anti-Human IL-8 (Cat. No. 554716) with recombinant human IL-8 (Cat. No. 554609) as the standard. This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. For detection of IL-8 in serum or plasma, the Human IL-8 BD OptEIA™ ELISA Set (Cat. No. 555244) or OptEIA™ ELISA Kit (Cat. No. 550999) is recommended.

### Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
555058	PE Mouse IgG2b, $\kappa$ Isotype Control	0.1 mg	27-35
555397	FITC Mouse Anti-Human CD14	100 Tests	M5E2
554609	Recombinant Human IL-8	20 $\mu\text{g}$	(none)
554715	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
554717	Purified Mouse Anti-Human IL-8	0.1 mg	G265-8
550419	Purified Mouse Anti-Human IL-8	0.25 mg	G265-8
554718	Biotin Mouse Anti-Human IL-8	0.5 mg	G265-8
554716	Purified Mouse Anti-Human IL-8	0.5 mg	G265-5
555244	Human IL-8 ELISA Set	20 Plate(s)	(none)
550999	Human IL-8 ELISA Kit II	2 Plate(s)	(none)

### 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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

### References

Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. *Cytokine*. 1989; 1(1):2-13. (Biology)  
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)