Technical Data Sheet

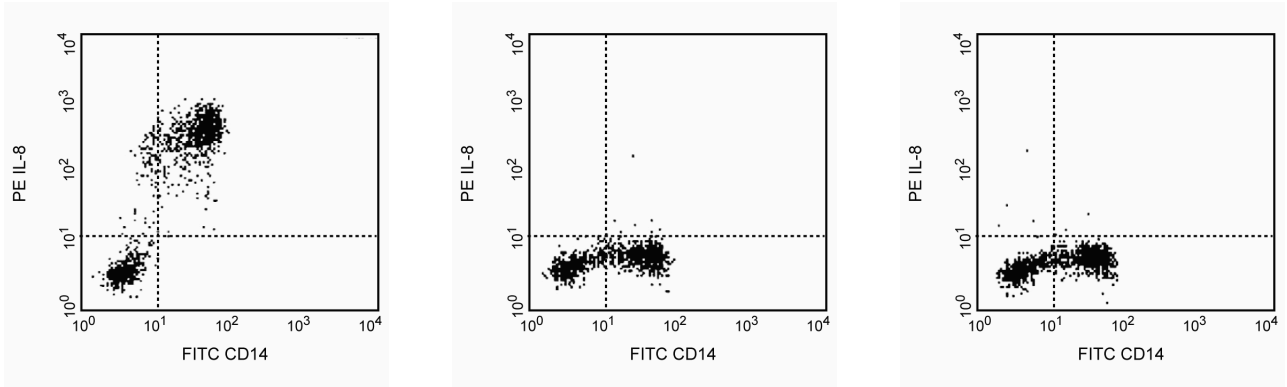
Purified Mouse Anti-Human IL-8

Product Information

Material Number:	**554717**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G265-8
Immunogen:	Recombinant Human IL-8
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The G265-8 antibody reacts with both the 72 and 77 amino acid forms of human interleukin-8 (IL-8). The immunogen used to produce the G265-8 hybridoma was E. coli-expressed recombinant human IL-8.



Expression of IL-8 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 6 hours with LPS (10 ng/ml final concentration) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC-mouse anti-human CD14 antibody (FITC-M5E2, Cat. No. 555397), fixed, permeabilized, and subsequently stained with 0.25 µg of PE-mouse anti-human IL-8 antibody (PE-G265-8, Cat. No. 554720) following the BD Pharmingen 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-G265-8 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 unlabelled G265-8 antibody (2.5 µg, Cat. No. 554717; right panel) prior to staining with the PE-G265-8 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (center panel) and unlabelled antibody (right panel) blocking specificity controls.*

Preparation and Storage

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

Store undiluted at 4°C.

Application Notes

Application

Intracellular block/flow cytometry	Routinely Tested
Immunocytochemistry (cytospins)	Tested During Development
ELISA	Tested During Development
Flow cytometry	Tested During Development

Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified G265-8 antibody (Cat. No. 554717) can be used as a blocking control to demonstrate specificity of IL-8 staining by PE-G265-8 antibody (Cat. No. 554720). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1-10 µg of unlabeled G265-8 antibody (Cat. No. 554717) for 20 minutes at 4°C, prior to staining with PE-G265-8 antibody (e.g., 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.

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ELISA Detection: The biotinylated G265-8 antibody (Cat. No. 554718) is useful as a detection antibody for a sandwich ELISA for measuring human IL-8 protein levels. Biotinylated G265-8 antibody can be paired with the purified G265-5 antibody (Cat. No. 554716) with recombinant human IL-8 (Cat. No. 554609) as the standard. For testing IL-8 in serum or plasma, our human IL-8 OptEIA™ ELISA Set (Cat. No. 555244) and Kit (Cat. No. 550999) are specially formulated and recommended.

Immunofluorescent Staining and Flow Cytometric Analysis: The G265-8 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate human IL-8 producing cells within mixed cell populations. The PE-conjugated G265-8 antibody (Cat. No. 554720) is especially suitable for these experiments. 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 web site, www.bdbiosciences.com.

Immunocytochemical staining: The purified format of G265-8 antibody is useful for ICC staining with a DAB substrate. The Cat. No. 550419 is tested in the ICC application.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554720	PE Mouse Anti-Human IL-8	0.1 mg	G265-8
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555397	FITC Mouse Anti-Human CD14	100 tests	M5E2
554609	Recombinant Human IL-8	20 µg	(none)
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. IL-8 is protected under U.S. Patent Nos. 5,652,338 and 5,698,196.
4. 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.

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)