Technical Data Sheet

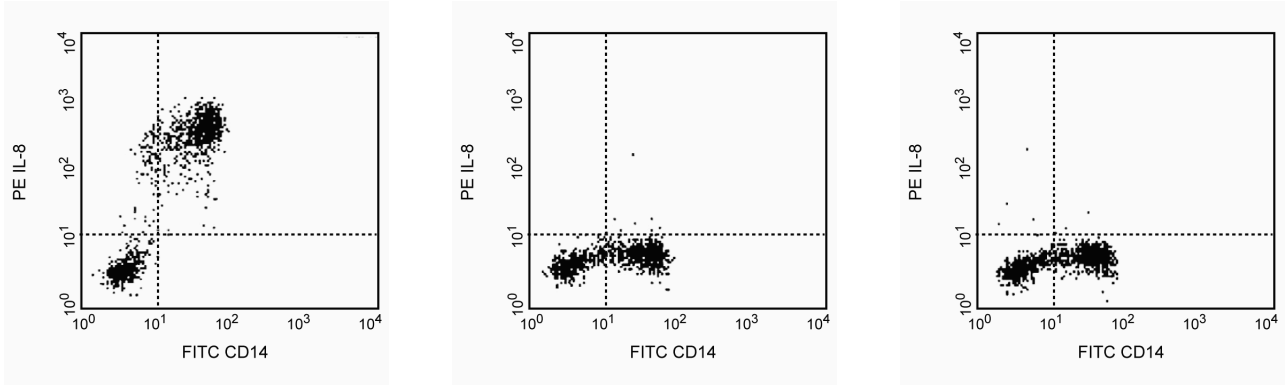
PE Mouse Anti-Human IL-8

Product Information

Material Number:	**554720**
Size:	0.1 mg
Concentration:	0.2 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 2 µM GolgiStop™ (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 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-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) and unlabelled antibody (right) blocking specificity controls.*

Preparation and Storage

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.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The PE-conjugated G265-8 antibody (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 (≤ 0.5 µg mAb/million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

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 unlabelled G265-8 antibody (Cat. No. 554717) 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 conjugated clone 27-35 (Cat. No. 555058).

Cytokine ICC: The G265-8 antibody is useful for immunocytochemical staining. Our Cat. No. 550419 is tested in the ICC application.

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ELISA Detection: The biotinylated G265-8 antibody (Catalog No. 554718) is useful as a detection antibody in 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. 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

Catalog Number	Name	Size	Clone
555058	PE Mouse IgG2b, κ Isotype Control	0.1 mg	27-35
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)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. IL-8 is protected under U.S. Patent Nos. 5,652,338 and 5,698,196.
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.

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)