Technical Data Sheet

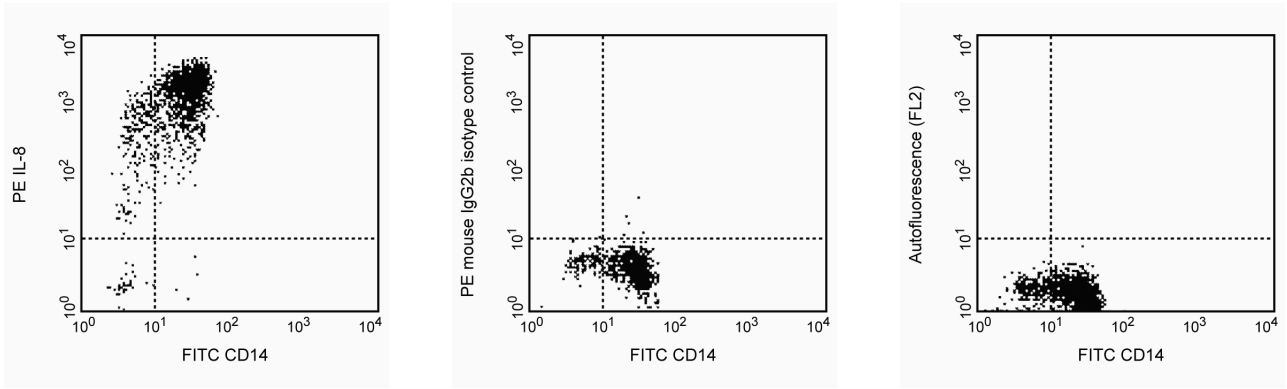
PE Mouse IgG2b, κ Isotype Control

Product Information

Material Number:	**555058**
Alternate Name:	anti-dansyl
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	27-35
Isotype:	Mouse (C.SW) IgG2b, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The mouse IgG2b, κ immunoglobulin isotype control monoclonal antibody 27-35 is specific for the hapten dansyl (5-[dimethylamino] naphthalene-1-sulfonyl). This hapten is not expressed on human cells or human cell lines. The 27-35 immunoglobulin was selected as an isotype control following testing which demonstrated low background staining on a variety of mouse and human tissues.



Expression of human IL-8 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 6 hours with LPS (1.0 µg/ml final concentration) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with 0.25 µg of FITC-mouse anti-human CD14 monoclonal antibody (FITC-M5E2, Cat. No. 555397), fixed, permeabilized, and subsequently stained with either 0.125 µg of PE-anti-human IL-8 (Cat. No. 554720; left panel), or 0.125 µg of PE-mouse IgG2b (Cat. No. 555058; middle panel), following BD PharMingen staining protocol. The data reflect gating on monocytes, based on forward and side scattered light signals. The quadrant markers for the bivariate dot plot were set based on autofluorescence controls (right panel).*

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

Flow cytometry	Routinely Tested
Isotype control	Routinely Tested
Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE- and FITC-conjugated 27-35 immunoglobulin (Cat. No. 555057; 555058) are suitable mouse IgG2b isotype controls for assessing the level of background staining on paraformaldehyde fixed/saponin-permeabilized rat or human cells for flow cytometric analysis. Use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells) (see image, right panel). 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. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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

BD Biosciences. Techniques for Immune Function Analysis, Application Handbook 1st Edition. 2003; Available: http://www.bdbiosciences.com/pdfs/manuals/02-8100055-21A1rr.pdf 2007, Jan. 25. (Methodology)

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)