Technical Data Sheet

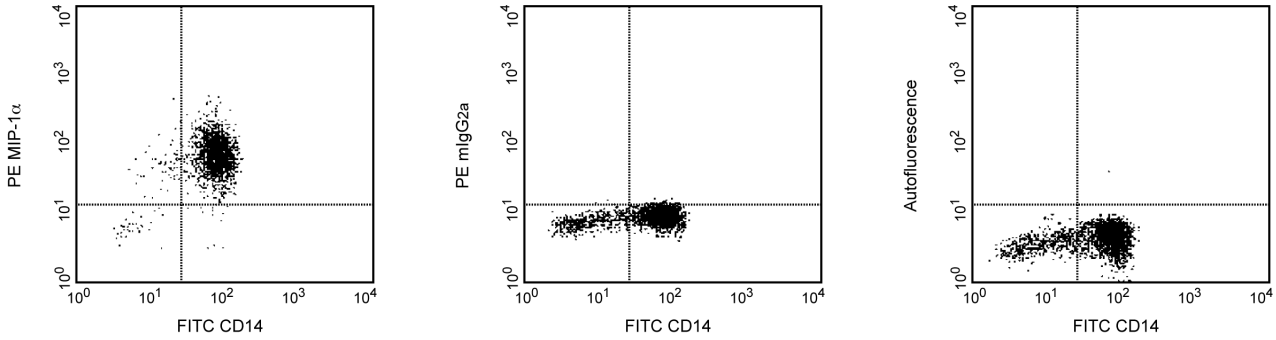
PE Mouse IgG2a, κ Isotype Control

Product Information

Material Number:	**559319**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	G155-178
Immunogen:	TNP-keyhole limpet hemocyanin
Isotype:	Mouse (BALB/c) IgG2a, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The G155-178 clone has an unknown specificity. Trinitrophenol (TNP), the immunogen, is a hapten not expressed on human, mouse, rat or non-human primate cells. In the absence of specific binding, this antibody may bind non-specifically to immunoglobulin Fc receptors. The immunoglobulin secreted by the G155-178 hybridoma was selected as an mouse IgG2a, κ isotype control following screening for low background binding on a variety of mouse and human tissues.



Expression of human MIP-1α by stimulated CD14+ human lymphocytes. *Human PBMC were stimulated for 6 hours with LPS (100 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 monoclonal antibody (FITC-M5E2, Cat. No. 555397), fixed permeabilized, and subsequently stained with either PE-anti-human MIP-1α (Cat. No. 554730, top panel), or PE-mouse IgG2aκ (Cat. No. 559319; middle panel), by following the Pharmingen staining protocols. The data reflect gating on monocytes, based on forward and scattered light signals. The quadrant markers for the bivariate dot plot were set based on autofluorescence controls (bottom 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-conjugated G155-178 immunoglobulins are a suitable mouse IgG2a, κ isotype control for assessing the level of background staining on paraformaldehyde fixed/saponin-permeabilized rat or human cells for flow cytometric analysis. 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. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

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This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized. Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	100 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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: Flow cytometry)