Technical Data Sheet

FITC Rat IgG1, κ Isotype Control

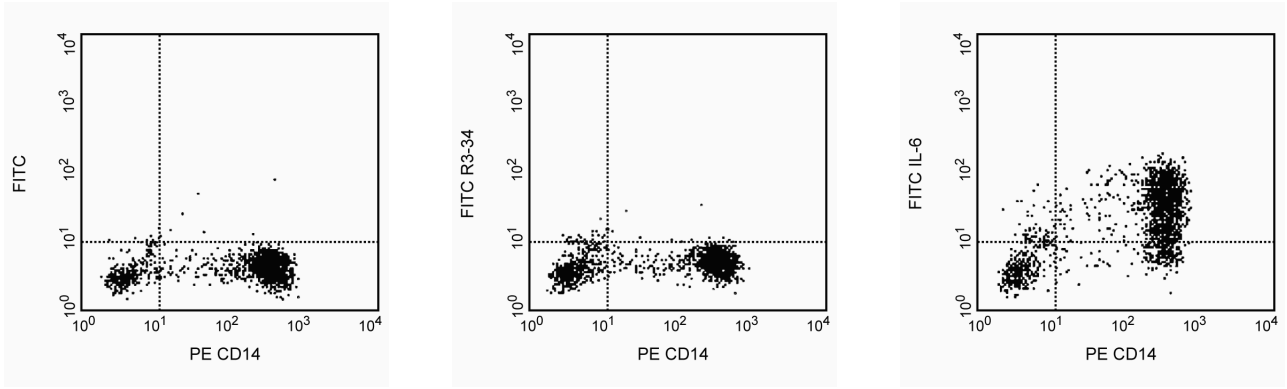
Product Information

Material Number:	**554684**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	R3-34
Isotype:	Rat IgG1, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The immunoglobulin from the R3-34 hybridoma was identified as a non-reactive clone, following immunization with mouse Ig. The R3-34 immunoglobulin was selected as an isotype control following screening for low background on a variety of mouse and human tissues.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of IL-6 by stimulated CD14+ human monocytes. *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 PE-mouse anti-human CD14 monoclonal antibody (PE-M5E2, Cat. No. 555398), fixed, permeabilized, and subsequently stained with either 0.25 µg of FITC-Rat IgG1 isotype control immunoglobulin (FITC-R3-34; Cat. No. 554684; middle panel) or with 0.25 µg of FITC-rat anti-human IL-6 antibody (FITC-MQ2-13A5; Cat. No. 554544; right panel), following Pharmingen's staining protocol. Left panel portrays autofluorescence detected by the FL1 PMT versus CD14-PE staining. The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, the binding by FITC-MQ2-13A5 antibody was blocked by each of the following: 1) preincubation of the fluorochrome-conjugated antibody with recombinant human IL-6 (Cat. No. 550071; data not shown) and by 2) preincubation of the fixed/permeabilized cells with unlabeled MQ2-13A5 antibody (Cat. No. 554542; data not shown) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the ligand blocking and unlabeled antibody 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 FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

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

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Recommended Assay Procedure:

The FITC-, PE- and APC-conjugated R3-34 immunoglobulins (Cat. No. 554684; 554685; 554686) are suitable rat IgG1 isotype controls for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse and human cells for flow cytometric analysis. Use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg antibody/1 million cells (see image, right panel). For specific methodology, see online protocols or chapter 4 on intracellular staining in Techniques for Immune Function Analysis Application Handbook 1st Edition, both of which are located at our website at www.bdbiosciences.com. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(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. 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

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: IC/FCM Block)