Technical Data Sheet

FITC Rat IgG2a, κ Isotype Control

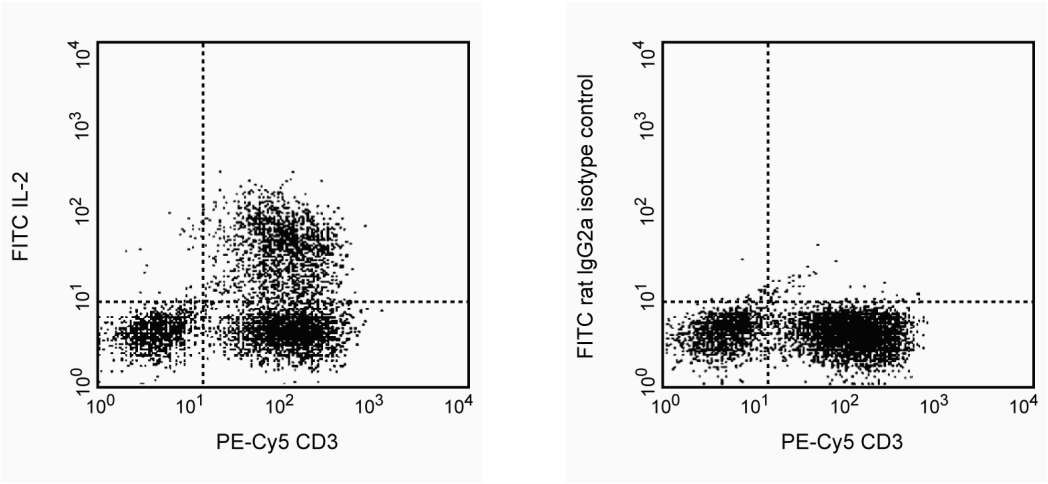
Product Information

Material Number:	**554688**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	R35-95
Immunogen:	Mouse Pooled Immunoglobulin
Isotype:	Rat (LOU) IgG2a κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The R35-95 hybridoma was generated by hybridization of Y3 myeloma cells with spleen cells from LOU rats immunized with mouse immunoglobulins. The R35-95 hybridoma produces rat IgG2a, κ immunoglobulin that has no measurable reactivity with mouse immunoglobulins. The R35-95 immunoglobulin was selected as an isotype control following screening for low background binding 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-2 by stimulated CD3+ human PBMC. *Human PBMC were stimulated for 6 hours with PMA (Sigma) and calcium ionophore A23187 (Sigma) in the presence of GolgiStop™ (2 mM final concentration; Cat. No. 554714). The PBMC were stained with PE-Cy5-anti-CD3 (PE-CY5 UCHT1, Cat. No 555334), fixed, permeabilized, and subsequently stained with 0.25 mg of FITC-Rat IgG2a, κ anti-human IL-2 antibody (FITC-MQ1-17H12, Cat. No 554565; left panel) or 0.25 mg FITC-R35-95 isotype control immunoglobulin (FITC-R35-95, Cat. No. 554688; right panel) using Pharmingen's staining protocol. To demonstrate specificity of staining, the binding of FITC-MQ1-17H12 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant human IL-2 (Cat. No.554603; data not shown), and by preincubation of the fixed/permeabilized cells with an excess of the unlabelled MQ1-17H12 antibody (Cat. No. 554563; data not shown) prior to staining with the FITC-MQ1-17H12 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking and unlabelled 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.

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Application Notes

Application

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

Recommended Assay Procedure:

Immunofluroescent Staining and Flow Cytometric Analysis: The FITC-R35-95 immunoglobulin (Cat. 554688) is a suitable rat IgG2a κ isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells for flow cytometric analysis. Use at comparable concentrations to antibody of interest (e.g., 0.5 mg mAb/1 million cells). See image, right panel. 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.

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)
554714	BD Cytofix/Cytoperm Fixation/Permeablization Kit	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)