Technical Data Sheet

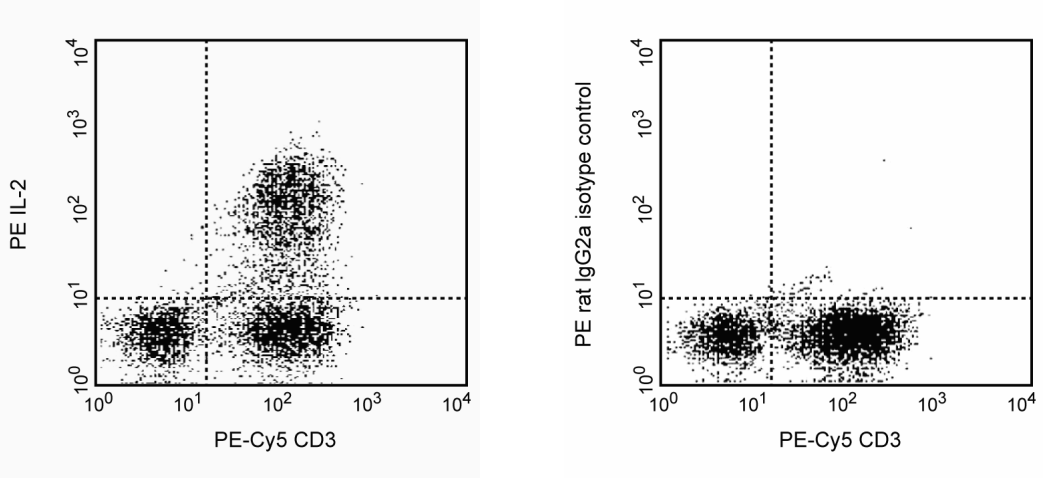
PE Rat IgG2a κ Isotype Control

Product Information

Material Number:	**559317**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	R35-95
Immunogen:	Mouse Pooled Immunoglobulin
Isotype:	Rat (LOU) IgG2a, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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.



Expression of IL-2 by stimulated CD3+ human PBMC. *Human PBMC were stimulated for 6 hours with PMA (Sigma, Cat. #P-8139) and calcium ionophore A23187 (Sigma, Cat. #C-9275) in the presence of BD GolgiStop™ (2 µl final concentration; Cat. No.554724). The PBMC were stained with PE-Cy5-anti-CD3 (PE-Cy5-UCHT1, Cat. No. 555334), fixed, permeabilized, and subsequently stained with 20 µl of PE-Rat IgG2a κ anti-human IL-2 antibody (PE-MQ1-17H12, Cat. No. 559334; left panel) or 20 µl PE-R35-95 isotype control immunoglobulin (PE-R35-95, Cat. No. 559317; right panel) by following the Usage section above and Pharmingen's staining protocol. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control.*

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
Isotype control	Routinely Tested

Neutralization Activity:

Neutralization: The NA/LE™ R35-95 (Cat. No. 554687) is suitable as an isotype control for rat IgG2a κ neutralizing antibodies.

Recommended Assay Procedure:

I/C Flow Cytometry: The PE-conjugated R35-95 immunoglobulins (Cat. No. 559317) is a suitable rat IgG2a κ isotype controls for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells for flow cytometric analysis.

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Important Note: 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 cells. BD Perm/Wash™ Buffer (Cat. No 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the Protocol section below.

Protocol

1. Resuspend 1 x 10^6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X Perm/Wash Buffer (Cat. No 554723).
2. Incubate the cell suspension for 15 minutes (at RT or 4°C).
3. Wash twice in 100 µl of 1X Perm/Wash Buffer (Cat. No 554723).

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. An isotype control should be used at the same concentration as the antibody of interest.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
6. 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.
7. 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. (Biology)