Technical Data Sheet

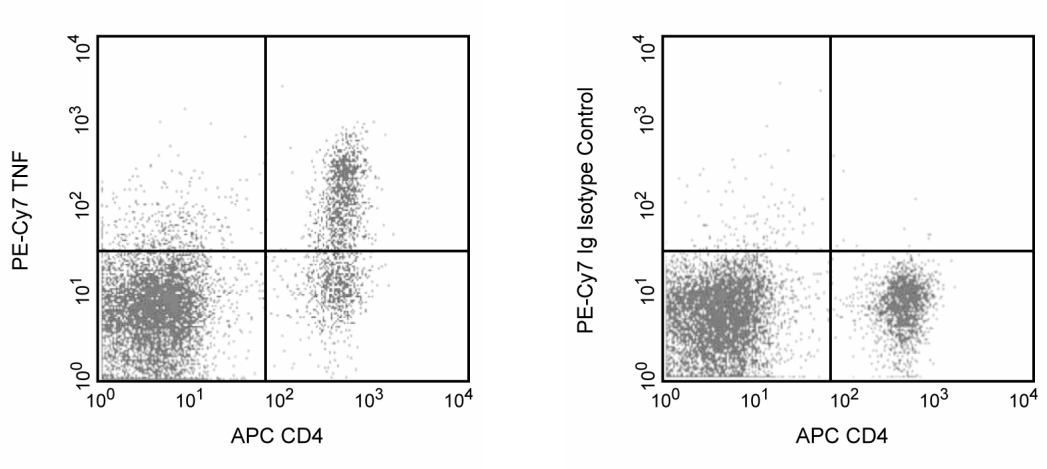
PE-Cy™7 Rat IgG1 κ Isotype Control

Product Information

Material Number:	**557645**
Size:	0.1 mg
Concentration:	0.2 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.



Expression of TNF by stimulated CD4+ and CD4- BALB/c spleen cells. *Splenocytes from BALB/c mice were stimulated for 4 hours with PMA (5 ng/ml, Sigma, Cat. No. P-8139) and Ionomycin (500 ng, Sigma, P-8139) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with APC-conjugated rat anti-mouse CD4 (APC-RM4-5, Cat. No. 553051) and either rat anti-mouse TNF antibody (PE-Cy7-MP6-XT22, Cat. No. 557644) (left panel) or immunoglobulin isotype control (PE-Cy7-R3-34, Cat. No. 557645), (right panel) by using Pharmingen's staining protocol. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 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
Neutralization	Tested During Development

Recommended Assay Procedure:

Immunofluorescent Staining for Intracellular Cytokines: The PE-Cy7-conjugated-R3-34 immunoglobulin (Cat. No. 557645) is a suitable rat IgG1 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. The intracellular cytokine staining technique and use of blocking controls are decribed in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our website, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook. R3-34 is suitable as an isotype control for rat IgG1 neutralizing antibodies. Cat. No. 554682, No azide, low endotoxin formulation is recommended for neutralization assays.

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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. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
4. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
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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)