

## Technical Data Sheet

## Alexa Fluor® 647 Rat IgG1, κ Isotype Control

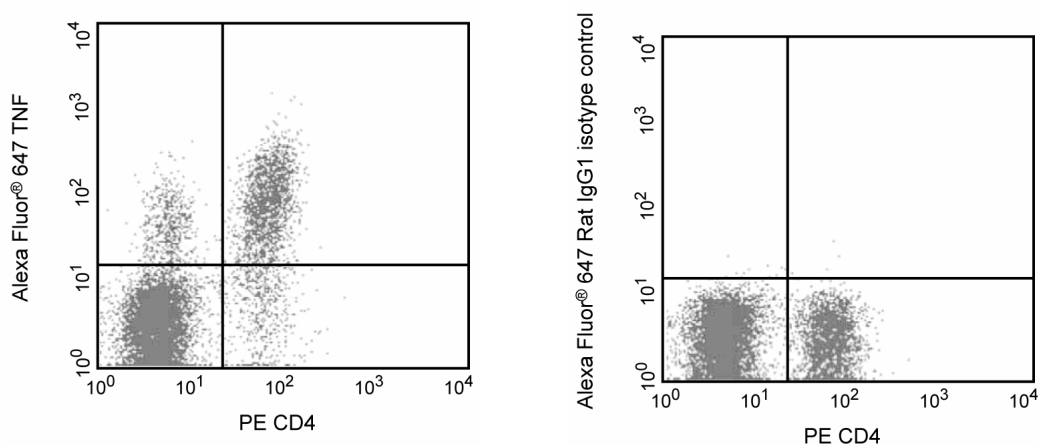
## Product Information

Material Number:	557731
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.

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 TNF by stimulated CD4<sup>+</sup> and CD4<sup>-</sup> 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, I-0634) in the presence of Brefeldin A (BD GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized, and stained with PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553048) and either rat anti-mouse TNF antibody (Alexa Fluor® 647-MP6-XT22, Cat. No. 557730) (left panel) or immunoglobulin isotype control (Alexa Fluor® 647-R3-34, Cat. No. 557731) (right panel) using the BD Biosciences Pharmingen staining protocol. Dot plots were derived from gated events with the forward and side light scatter characteristics of lymphocytes. 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was 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
Flow cytometry	Routinely Tested
Isotype control	Routinely Tested

## Recommended Assay Procedure:

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**Immunofluorescent Staining for Intracellular Cytokines:** The Alexa Fluor® 647-conjugated-R3-34 immunoglobulin (Cat. No. 557731) is a

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suitable rat IgG1 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. For specific methodology, please visit our web site, [www.bdbiosciences.com](http://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

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554715	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555028	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiPlug)	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/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. 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.
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

### 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)