Technical Data Sheet

Alexa Fluor® 488 Rat IgG1, κ Isotype Control

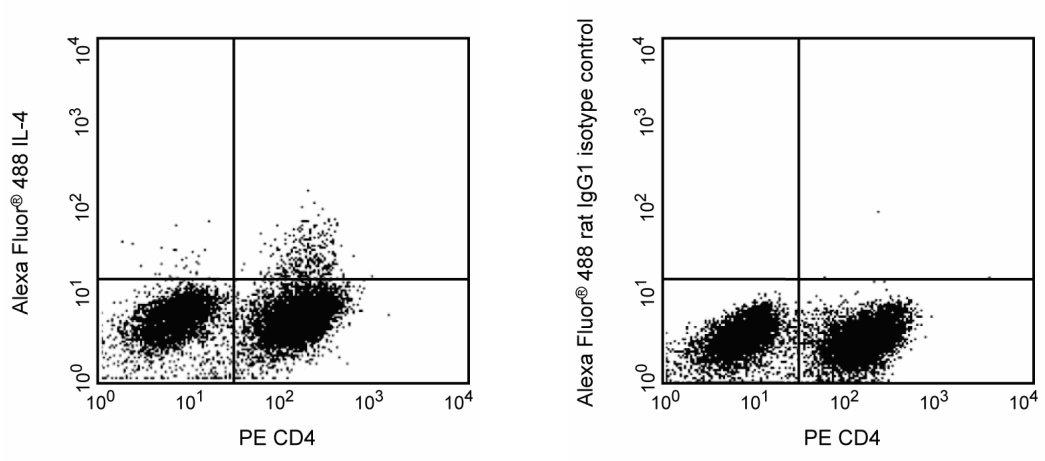
Product Information

Material Number:	**557856**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	R3-34
Isotype:	Rat IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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-4 by stimulated human peripheral blood mononuclear cells. *Human peripheral blood mononuclear cells were stimulated with plate-bound anti-human CD3 (HIT3a, 10 µg/ml, Cat. No. 555336) and soluble anti-CD28 (CD28.2, 2 µg/ml, Cat. No. 555725) antibody in the presence of recombinant human IL-2 (10 ng/ml, Cat. No. 554603) and IL-4 (25 ng/ml, Cat. No. 554605) for 2 days. The cells were subsequently washed and expanded in IL-2 and IL-4 for 3 days. Following expansion the cells were washed and 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 (GolgiPlug, Cat. No. 555029). Following incubation the cells were harvested and stained with either rat anti-human IL-4 (Alexa Fluor® 488-MP4-25D2) (left panel) or immunoglobulin isotype control (Alexa Fluor® 488-R3-34, Cat. No. 557856) (right panel) by using Pharmingen's staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 488 was blocked by preincubation of the fixed/permeabilized cells with an excess of unlabeled MP4-25D2 (5 µg, Cat. No. 554482, data not shown) prior to staining. 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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 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
Isotype control	Routinely Tested

Recommended Assay Procedure:

Recommended Assay Procedure:

Immunofluorescent Staining for Intracellular Cytokines: The FITC-, PE-, APC-, PE-Cy7-, Alexa Fluor® 488-, and Alexa Fluor®

BD Biosciences

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647-conjugated-R3-34 immunoglobulin (Cat. No. 554684, 554685, 557856, 557865, 554686, and 557645) is a suitable rat IgG1 κ isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells for flow cytometric analysis. Use at 5 µl/test. The intracellular cytokine staining technique and use of blocking controls are described 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.

An isotype control should be used at the same concentration as the antibody of interest.

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
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.
8. 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.
9. 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)