

Technical Data Sheet

Alexa Fluor® 647 Rat IgG2a, κ Isotype Control

Product Information

Material Number:	557906
Size:	100 tests
Vol. per Test:	5 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.

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.

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
Isotype control	Routinely Tested

Neutralization Activity:

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

Recommended Assay Procedure:

An isotype control should be used at the same concentration as the antibody of interest (e.g., ≤ 1 µg/million cells for flow cytometry).

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 with Cytofix/Cytoperm Kit (Cat. No. 554714). BD Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the USAGE section below.

USAGE for Intracellular Staining

Resuspend 1 × 10⁶ fixed and permeabilized cells in 5 µl of the pre-titered antibody solution and 45 µl of 1X Perm/Wash Buffer (Cat. No. 554723).

Incubate the cell suspension for 15 minutes (at RT or 4°C).

Wash twice in 100 µl of 1X Perm/Wash Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	100 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit	250 tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10⁶ 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.

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4. 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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. (Biology)