Flow Cytometry Secondary Reagent
Rat Anti-mouse IgG_{2B}-CFS

Catalog Number: F0134
Lot Number: ACNM02
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated rat monoclonal anti-mouse IgG_{2B}: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 332723

Isotype: rat IgG_1

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

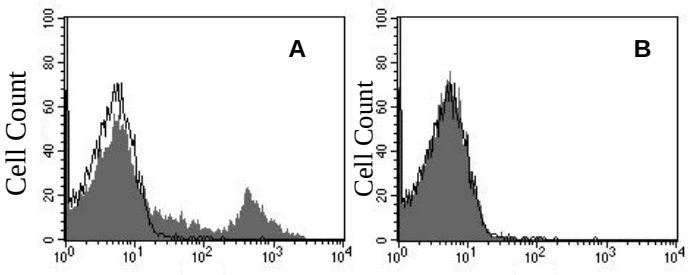
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

This reagent is designed for use as a secondary developing reagent in immunofluorescent assays, such as flow cytometry, where the primary antibody does not have a fluorescent reporter molecule, is of mouse origin, and is of the IgG_{2B} class.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with a cocktail of mouse IgG_{2B} and IgG_3. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to CFS fluorochrome. Detection of a mouse IgG_{2A} primary antibody is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



Human peripheral blood lymphocytes were stained with A) Anti-human CD8α (Catalog # MAB1509, mIgG2B, filled histogram) or isotype control (Catalog # MAB0041, open histogram), or B) Anti-human CD4 (Catalog # MAB3791, mIgG2A, filled histogram) or isotype control (Catalog # MAB0041, open histogram), followed by CFS-conjugated Rat Anti-mIgG2B isotype-specific secondary antibody (Catalog # F0134).

Specificity

The specificity of this antibody was confirmed by direct ELISA on mouse IgG_{2B} monoclonal antibodies, and it does not cross-react with IgG_1, IgG_{2A}, IgG_3, IgM, IgA, or IgE antibodies.

By flow cytometry, this isotype-specific secondary antibody detects primary antibodies of the mouse IgG_{2B} isotype, and does not detect primary antibodies of mouse IgG_1, IgG_{2A}, or IgG_3 class.

Sample Staining

1. Stain cells of interest (up to 1 x 10^6 cells) with a mouse IgG_{2B} primary antibody according to the antibody manufacturer's instructions.
2. After the recommended incubation period, wash the cells 3 times with a PBS buffer, such as Flow Cytometry Staining Buffer (Catalog # FC001).
3. Add 10 µL of rat anti-mouse IgG_{2B}-CFS to each sample.
4. Incubate the cells for 30 minutes in the dark, then wash 3 times as in step #2.
5. Resuspend the cells in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with mouse IgG_{2B} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

R&D Systems Inc.
1-800-343-7475