

Technical Data Sheet

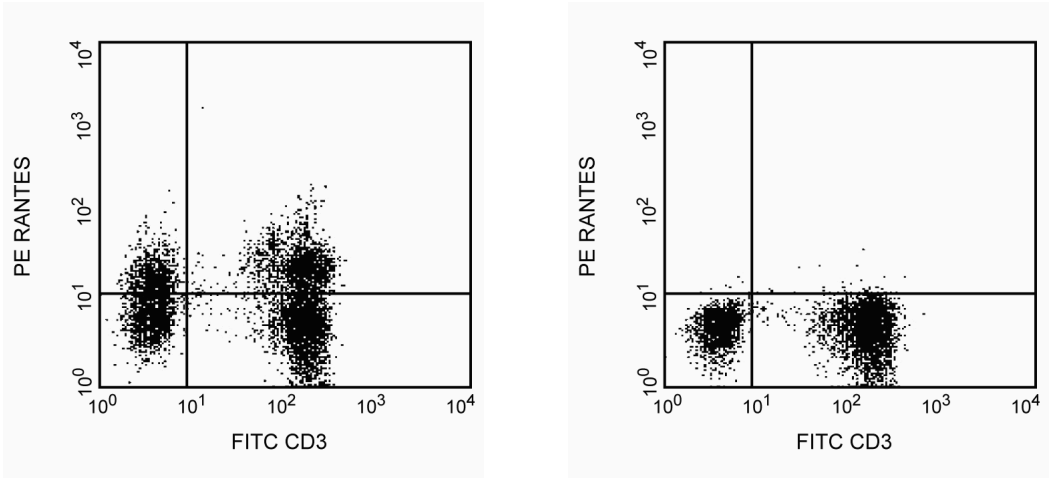
PE Mouse Anti-Human RANTES

Product Information

Material Number:	559322
Size:	100 tests
Vol. per Test:	20 µl
Clone:	2D5
Immunogen:	Recombinant human RANTES protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 2D5 antibody reacts with human Regulated upon Activation, Normal T cells Expressed and Secreted (RANTES). The immunogen used to generated this hybridoma was recombinant human RANTES protein.



Expression of RANTES by cultured human PBMC. Human PBMC were cultured for 24 hours in the presence of BD GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC-mouse anti-human CD3 antibody (FITC-HIT3a, Cat. No. 555339), fixed, permeabilized, and subsequently stained with 20 µl of PE-mouse anti-human RANTES antibody (Cat. No. 559322) by following the Usage section below and BD Biosciences Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-2D5 antibody was blocked by the preincubation of the unlabeled 2D5 antibody with fixed/permeabilized target cells prior to staining with the PE-2D5 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with unlabeled antibody blocking control (right panel).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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
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Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The PE-conjugated 2D5 antibody (Cat. No. 559322) can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate human RANTES producing cells within mixed cell populations. This 100 Test Size formulation of the PE-conjugated 2D5 antibody has been pre-titrated to assure effective intracellular detection of human RANTES using 20 µl/1 x 10⁶ cells.

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A useful control for demonstrating specificity of staining is to pre-block the fixed/permeabilized cells with unlabeled 2D5 antibody (Cat. No. 556859) prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control immunoglobulin for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-MOPC-21 immunoglobulin (Cat. No. 559320). For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

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. Perm/Wash. Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the Usage section.

Usage

1. Resuspend 1×10^6 fixed and permeabilized cells in 20 μ l of the pre-titered antibody solution and 30 μ l of 1x BD Perm/Wash. Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (at room temperature or 4°C).
3. Wash twice in 100 μ l of 1x BD Perm/Wash. Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
554723	Perm/Wash Buffer	100 ml	(none)

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- μ l 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/pharming/en/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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)

Sticherling M, Kupper M, Koltrowitz F, et al. Detection of the chemokine RANTES in cytokine-stimulated human dermal fibroblasts. *J Invest Dermatol*. 1995; 105(4):585-591. (Clone-specific)