Technical Data Sheet

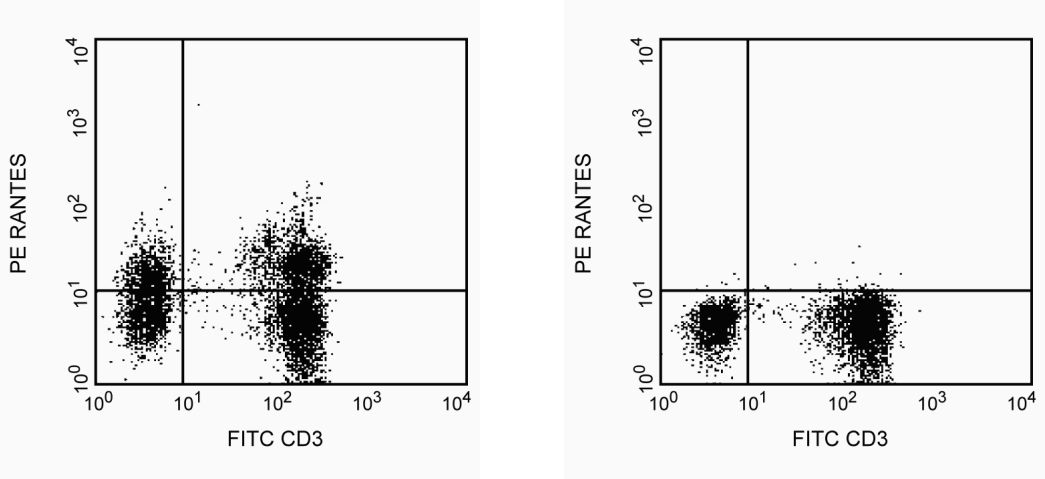
PE Mouse Anti-Human RANTES

Product Information

Material Number:	**554732**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	2D5
Immunogen:	Recombinant human RANTES protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2D5 antibody reacts with human **R**egulated upon **A**ctivation, **N**ormal **T** cells **E**xpressed and **S**ecreted (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 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 0.125 µg of PE-mouse anti-human RANTES antibody (PE-2D5, Cat. No. 554732) following 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

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The PE-conjugated 2D5 antibody (Cat. No. 554732) can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate human RANTES producing cells within mixed cell populations. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, visit the protocols section of our website, or the chapter on intracellular staining in the Immune Function Handbook, which is posted on our web site at www.bdbiosciences.com.

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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 paraformaldehydefixed/saponin-permeabilized human cells is the PE-MOPC-21 immunoglobulin (Cat. No. 554680); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	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/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.

References

Leonard EJ, Sylvester I, Yoshimura T, et al. *Current Protocols in Immunology.* John Wiley and Sons; 1995:6.12.1-6.12.26. (Methodology: Neutralization)

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: IC/FCM Block)

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)