

Technical Data Sheet

PE-Cy™5 Rat Anti-Mouse CD4

Product Information

Material Number:	561836
Alternate Name:	L3T4
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	RM4-5
Immunogen:	Mouse Thymocytes (BALB/c)
Isotype:	Rat (DA) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The RM4-5 clone has been reported to react with the CD4 (L3T4) differentiation antigen expressed on most thymocytes, subpopulations of mature T lymphocytes (i.e., MHC class II-restricted T cells, including most T helper cells and immunosuppressive regulatory T cells), and a subset of NK-T cells. CD4 has also been reported to be detected on pluripotent hematopoietic stem cells, bone marrow myeloid and B-lymphocyte precursors, intrathymic lymphoid precursors, and a subset of splenic dendritic cells. CD4 has been reported to be expressed on the plasma membrane of mouse egg cells and is involved in adhesion of the egg to MHC class II-bearing sperm. CD4 is an antigen coreceptor on the T-cell surface which interacts with MHC class II molecules on antigen-presenting cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase lck. Purified RM4-5 mAb has been reported to block the binding of FITC-conjugated anti-mouse CD4 clones GK1.5 and H129.19, but not the RM4-4 clone.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

Flow Cytometry: PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. **However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™.** Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553931	PE-Cy™5 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553061	FITC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11

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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
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.

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6. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
7. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
8. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
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10. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
11. An isotype control should be used at the same concentration as the antibody of interest.

References

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