

Technical Data Sheet

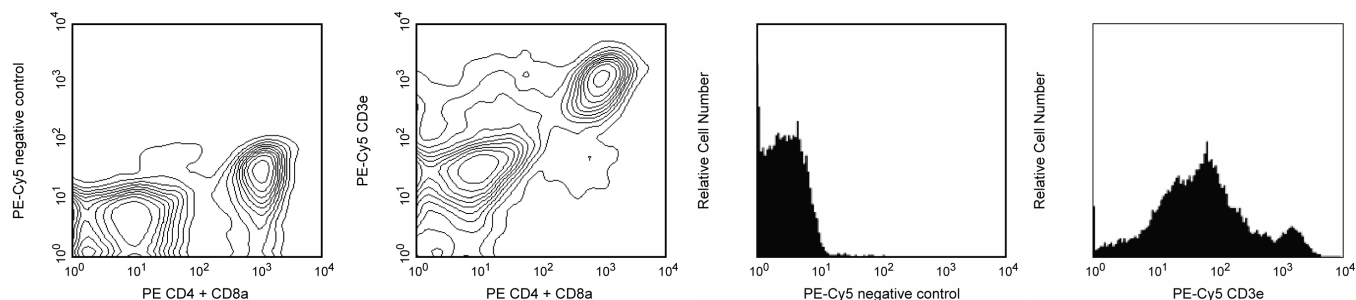
PE-Cy™5 Hamster Anti-Mouse CD3e

Product Information

Material Number:	553065
Alternate Name:	CD3ε chain
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	145-2C11
Immunogen:	H-2Kb specific cytotoxic T lymphocyte clone BM10-37
Isotype:	Armenian Hamster IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 145-2C11 antibody reacts with the 25-kDa ε chain of the T-cell receptor-associated CD3 complex, which is expressed on thymocytes, mature T lymphocytes, and NK-T cells. The cytoplasmic domain of CD3ε participates in the signal transduction events which activate several cellular biochemical pathways as a result of antigen recognition. Soluble 145-2C11 antibody can activate either unprimed (naive) or primed (memory/preactivated) T cells *in vivo* or *in vitro*, in the presence of Fc receptor-bearing accessory cells. In contrast, plate-bound 145-2C11 can activate T cells in the absence of accessory cells. Soluble 145-2C11 antibody has been reported to induce re-directed lysis of Fc receptor-bearing target cells by CTL clones and can also block lysis of specific target cells by antigen-specific CTL's. Under some conditions, T-cell activation by 145-2C11 antibody has been reported to result in apoptotic cell death. The 145-2C11 antibody does not cross-react with rat leukocytes and it has been reported that pre-incubation of thymus cell suspensions at 37°C for 2-4 hours prior to staining enhances the ability of anti-CD3ε and anti-αβ TCR mAbs to detect the T-cell receptor on immature thymocytes.



CD3e expression in spleen and thymus. BALB/c splenocytes were pre-incubated with Mouse BD Fc Block™ (Cat. No. 553142) then simultaneously stained with PE-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553049), PE-conjugated anti-mouse CD8a mAb 53-6.7 (Cat. No. 553033) and PE-Cy5-conjugated mAb 145-2C11 (middle left panel). BALB/c thymocytes were also stained with PE-Cy5-conjugated mAb 145-2C11 (far right panel) or unstained (middle right panel). Flow cytometry was performed on a BD FACScan™ flow cytometry system.

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

Recommended Assay Procedure:

Precautions for 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 2.4 G2) 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, or MRL mice as**

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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-Cy™5.5, APC and APC-Cy™7 tandem fluorochromes can alternatively be used on leukocytes from these mouse strains.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5
553033	PE Rat Anti-Mouse CD8a	0.2 mg	53-6.7
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
553973	PE-Cy™5 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
3. 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™.
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.
5. 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.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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