Technical Data Sheet

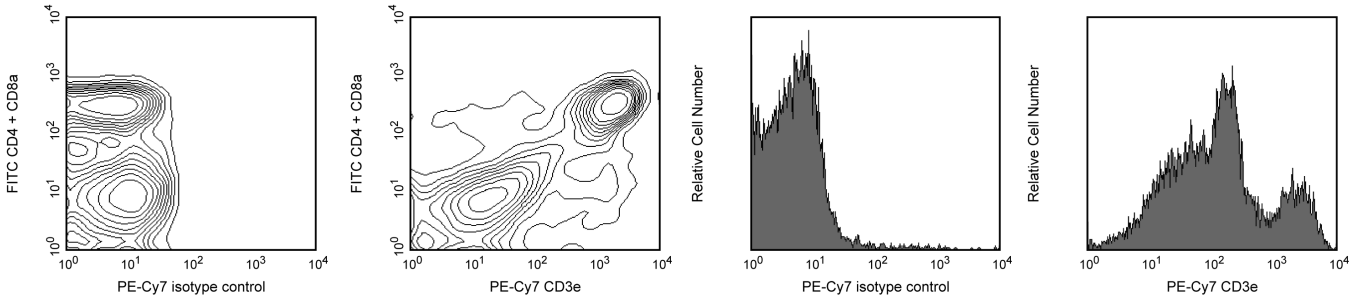
PE-Cy™7 Hamster Anti-Mouse CD3e

Product Information

Material Number:	**561100**
Alternate Name:	CD3ε chain
Size:	25 µg
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 CD3e 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 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 either PE-Cy7-conjugated hamster IgG1κ isotype control A19-3 (Cat. No. 552811; far left panel) or PE-Cy7-conjugated mAb 145-2C11 (middle left panel). BALB/c thymocytes were also stained with either PE-Cy7-conjugated isotype control (middle right panel) or PE-Cy7-conjugated mAb 145-2C11 (far right panel). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
552811	PE-Cy™7 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3
553033	PE Rat Anti-Mouse CD8a	0.2 mg	53-6.7
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5

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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. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
6. 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.
7. 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.
8. 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.
9. 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

Isakov N, Wange RL, Burgess WH, Watts JD, Aebersold R, Samelson LE. ZAP-70 binding specificity to T cell receptor tyrosine-based activation motifs: the tandem SH2 domains of ZAP-70 bind distinct tyrosine-based activation motifs with varying affinity. *J Exp Med.* 1995; 181(1):375-380. (Biology: Immunoprecipitation)

Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc Natl Acad Sci U S A.* 1987; 84(5):1374-1378. (Immunogen: Activation, Blocking, Cytotoxicity, Immunoprecipitation, Stimulation)

Nakano H, Yamazaki T, Miyatake S, Nozaki N, Kikuchi A, Saito T. Specific interaction of topoisomerase II beta and the CD3 epsilon chain of the T cell receptor complex. *J Biol Chem.* 1996; 271(11):6483-6489. (Biology: Immunoprecipitation)