Technical Data Sheet

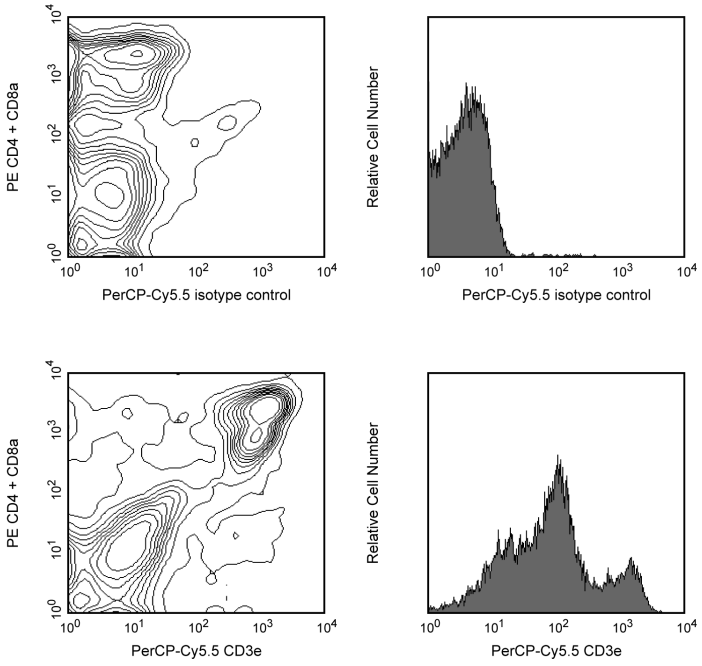
PerCP-Cy™5.5 Hamster Anti-Mouse CD3e

Product Information

Material Number:	**561108**
Alternate Name:	CD3; CD3 epsilon; Cd3e; CD3ε; T3e
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 monoclonal antibody specifically binds to the 25-kDa ε chain of the T-cell receptor-associated CD3 complex that is expressed on thymocytes, mature T lymphocytes, and NK-T cells. The cytoplasmic domain of CD3e participates in the signal transduction events that 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. Preincubation of thymus cell suspensions at 37°C for 2-4 hours prior to staining reportedly 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 PerCP-Cy5.5-conjugated hamster IgG1 isotype control mAb A19-3 (Cat. No. 550763, top left panel) or PerCP-Cy5.5 conjugated anti-mouse CD3e mAb 145-2C11 (bottom left panel). BALB/c thymocytes were also stained with either PerCP-Cy5.5 conjugated isotype control (top right panel) or PerCP-Cy5.5 conjugated mAb 145-2C11 (bottom right panel). Flow cytometry was performed on a BD FACSCalibur™ 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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

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Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
550763	PerCP-Cy™5.5 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5
553033	PE Rat Anti-Mouse CD8a	0.2 mg	53-6.7
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
3. PerCP is a photosynthetic accessory pigment from Glenodinium species of dinoflagellates, which is excited by the 488-nm light of an Argon ion laser and fluoresces at 675 nm. Therefore, PerCP-labelled antibodies can be used with FITC- and R-PE–labelled reagents in most single-laser flow cytometers with no significant spectral overlap of PerCP fluorescence with that of FITC or R-PE. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For third-color flow¬cytometric analysis using ≥25-mW laser power, we recommend PE-Cy5-, PE-Cy7–, or PerCP-Cy5.5-conjugated reagents.
4. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
5. 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/documents/hamster_chart_11x17.pdf.
6. 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.
7. 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.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Greimers R, Trebak M, Moutschen M, Jacobs N, Boniver J. Improved four-color flow cytometry method using fluo-3 and triple immunofluorescence for analysis of intracellular calcium ion ([Ca2+]i) fluxes among mouse lymph node B- and T-lymphocyte subsets. *Cytometry.* 1996; 23(3):205-217. (Methodology: Flow cytometry)

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)

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