Technical Data Sheet

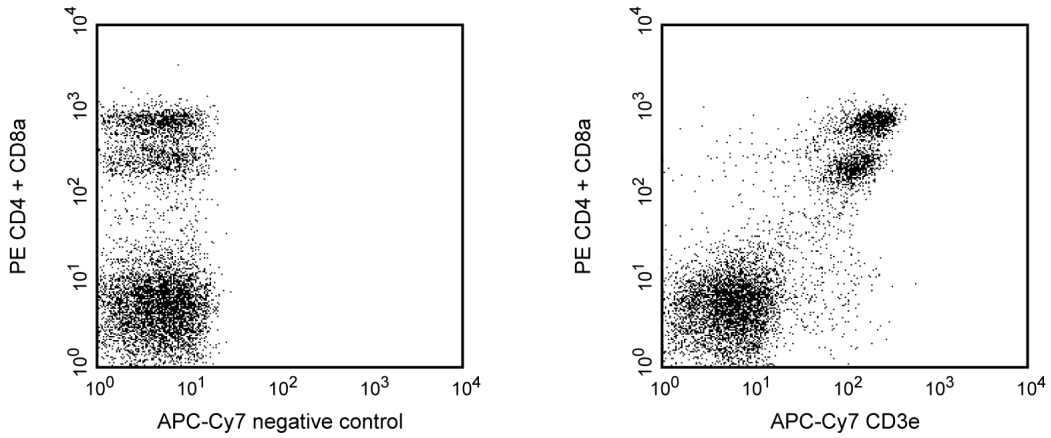
APC-Cy™7 Hamster Anti-Mouse CD3e

Product Information

Material Number:	**561042**
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.



Flow cytometric analysis of CD3e expression in mouse spleen. *C57BL/6 splenocytes were simultaneously stained with PE Rat Anti-Mouse CD4 mAb RM4-5 (Cat. No. 553048), PE Rat anti-mouse CD8a (Cat. No. 553032) and either APC-Cy™7 Hamster IgG1, κ Isotype Control (Cat. No. 557662, left panel) or APC-Cy™7 Hamster Anti-Mouse CD3e (Cat. No. 561042, right panel). Two-parameter flow cytometric dot plots showing the correlated expression of CD3e (or Ig Isotype control staining) versus CD8a and CD4 signals were derived from gated events with the forward and side light-scatter characteristics of intact splenocytes. Flow cytometry was performed on a BD FACSVantage SE™ 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 APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557662	APC-Cy™7 Hamster IgG1, κ Isotype Control	0.1 mg	A19-3
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5
553032	PE Rat Anti-Mouse CD8a	0.1 mg	53-6.7

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. 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).
5. 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.
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/documents/hamster_chart_11x17.pdf.
7. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
8. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Cy is a trademark of GE Healthcare.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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)

Kruisbeek AM, Shevach EM. Proliferative assays for T cell function. *Curr Protoc Immunol.* 2004; 3:3.12.1-3.12.14. (Methodology: Activation, Stimulation)

Kubo RT, Born W, Kappler JW, Marrack P, Pigeon M. Characterization of a monoclonal antibody which detects all murine alpha beta T cell receptors. *J Immunol.* 1989; 142(8):2736-2742. (Biology)

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)

Portoles P, Rojo J, Golby A, et al . Monoclonal antibodies to murine CD3 epsilon define distinct epitopes, one of which may interact with CD4 during T cell activation. *J Immunol.* 1989; 142(12):4169-4175. (Biology: Activation, Immunoprecipitation, Stimulation)

Shinkai Y, Alt FW. CD3 epsilon-mediated signals rescue the development of CD4+CD8+ thymocytes in RAG-2-/- mice in the absence of TCR beta chain expression. *Int Immunol.* 1994; 6(7):995-1001. (Biology: Activation, Stimulation)

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