

Technical Data Sheet

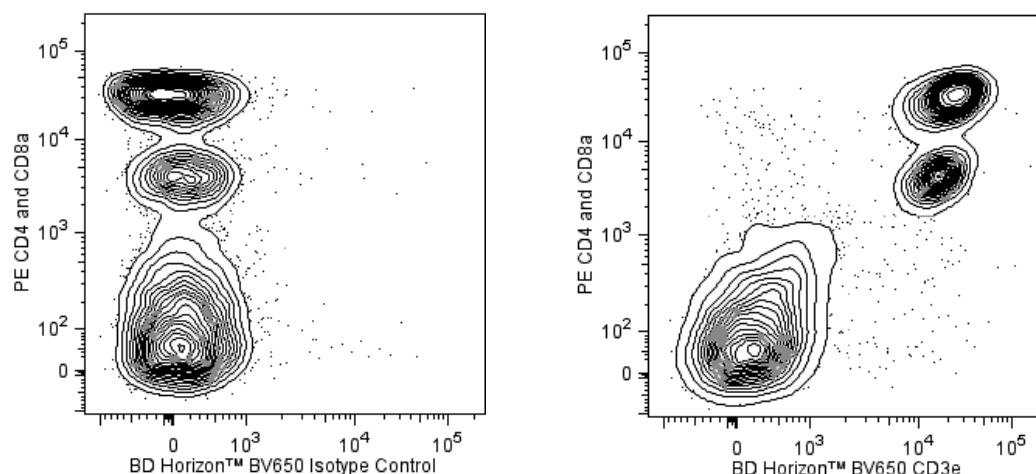
BV650 Hamster Anti-Mouse CD3e**Product Information**

Material Number:	564378
Alternate Name:	CD3; CD3 epsilon; Cd3e; CD3ε; T3e
Size:	50 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 BSA and ≤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.

The antibody was conjugated to BD Horizon BV650 which is part of the BD Horizon Brilliant™ Violet family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg. 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two-color flow cytometric analysis of CD3e expression on mouse splenocytes. Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with PE Rat Anti-Mouse CD4 (Cat. No. 553048/553049/561837) and PE Rat Anti-Mouse CD8a (Cat. No. 553033/553032/561095) antibodies and either BD Horizon™ BV650 Armenian Hamster IgG1, κ Isotype Control (Cat. No. 564388; Left Panel) or BD Horizon BV650 Hamster Anti-Mouse CD3e antibody (Cat. No. 564378; Right Panel). Two-color flow cytometric contour plots showing the correlated expression of CD3e (or Ig Isotype control staining) versus CD4 and CD8a were derived from gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometric analysis was performed using a BD LSR™ II Flow Cytometry System.

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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 BD Horizon™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

Application Notes

Application

Flow cytometry

Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)
564388	BV650 Hamster IgG1, κ Isotype Control	50 µg	A19-3
555899	Lysing Buffer	100 ml	(none)
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5
561837	PE Rat Anti-Mouse CD4	25 µg	RM4-5
553033	PE Rat Anti-Mouse CD8a	0.2 mg	53-6.7
553032	PE Rat Anti-Mouse CD8a	0.1 mg	53-6.7
561095	PE Rat Anti-Mouse CD8a	25 µg	53-6.7
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2

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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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- 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, Flow cytometry, Immunoprecipitation, Inhibition, Stimulation)
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