

## Technical Data Sheet

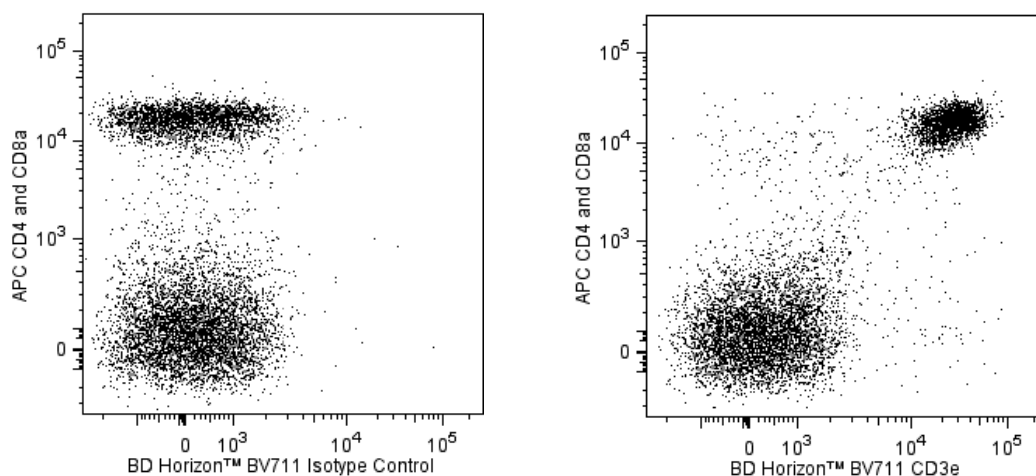
**BV711 Hamster Anti-Mouse CD3e****Product Information**

<b>Material Number:</b>	<b>563123</b>
<b>Alternate Name:</b>	CD3; CD3 epsilon; Cd3e; CD3ε; T3e
<b>Size:</b>	50 µg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	145-2C11
<b>Immunogen:</b>	H-2Kb specific cytotoxic T lymphocyte clone BM10-37
<b>Isotype:</b>	Armenian Hamster IgG1, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	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™ BV711 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 711-nm. BD Horizon™ BV711 can be excited by the violet laser and detected in a filter used to detect Cy™5.5 / Alexa Fluor® 700-like dyes (eg, 712/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there may be moderate spillover into the Alexa Fluor® 700 and PerCP-Cy™5.5 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



**Two-color flow cytometric analysis of CD3e expressed on mouse splenocytes.** BALB/c splenic leucocytes were stained with APC Rat Anti-Mouse CD4 (Cat. No. 553051/561091) and APC Rat Anti-Mouse CD8a (Cat. No. 553035/561093) antibodies and either BD Horizon™ BV711 Armenian Hamster IgG1, κ Isotype Control (Cat. No. 563128; Left Panel) or BD Horizon™ BV711 Hamster Anti-Mouse CD3e antibody (Cat. No. 563123; Right Panel). The two-color flow cytometric dot plots show CD3 (or Ig Isotype Control staining) versus CD4 and CD8 derived from events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometry 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™ BV711 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV711 were removed.

## Application Notes

### Application

Flow cytometry

Routinely Tested

## Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563128	BV711 Hamster IgG1, κ Isotype Control	50 µg	A19-3
553051	APC Rat Anti-Mouse CD4	0.1 mg	RM4-5
561091	APC Rat Anti-Mouse CD4	25 µg	RM4-5
553035	APC Rat Anti-Mouse CD8a	0.1 mg	53-6.7
561093	APC Rat Anti-Mouse CD8a	25 µg	53-6.7
555899	Lysing Buffer	100 ml	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Cy is a trademark of Amersham Biosciences Limited.
9. Brilliant Violet™ 711 is a trademark of Sirigen.
10. 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](http://www.bdbiosciences.com/documents/hamster_chart_11x17.pdf).

## References

Castro JE, Listman JA, Jacobson BA, et al. Fas modulation of apoptosis during negative selection of thymocytes. *Immunity*. 1996; 5(6):617-627. (Clone-specific: Activation, Apoptosis)

Duke RC, Cohen JJ, Boehme SA, et al. Morphological, biochemical, and flow cytometric assays of apoptosis. In: Coligan J, Kruisbeek AM, Margulies D, Shevach EM, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1995:3.17.1-3.17.33. (Methodology: Activation, Apoptosis)

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)

Kruisbeek AM, Shevach EM. Proliferative assays for T cell function. *Curr Protoc Immunol*. 2004; 1:3.12.1-3.12.14. (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. (Clone-specific: Activation, Flow cytometry, Immunoprecipitation, Stimulation)

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)

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. (Clone-specific: Functional assay, Stimulation)

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. (Clone-specific: Blocking, Cytotoxicity, Immunoprecipitation, Radioimmunoassay)

Radvanyi LG, Mills GB, Miller RG. Religation of the T cell receptor after primary activation of mature T cells inhibits proliferation and induces apoptotic cell death. *J Immunol*. 1993; 150(12):5704-5715. (Clone-specific: Activation, Apoptosis)

Salvadori S, Gansbacher B, Pizzimenti AM, Zier KS. Abnormal signal transduction by T cells of mice with parental tumors is not seen in mice bearing IL-2-secreting tumors. *J Immunol*. 1994; 153(11):5176-5182. (Clone-specific: Activation, Calcium Flux, Flow cytometry, Western blot)

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

Ucker DS, Meyers J, Obermiller PS. Activation-driven T cell death. II. Quantitative differences alone distinguish stimuli triggering nontransformed T cell proliferation or death. *J Immunol*. 1992; 149(5):1583-1592. (Clone-specific: Activation, Apoptosis)

Wang R, Murphy KM, Loh DY, Weaver C, Russell JH. Differential activation of antigen-stimulated suicide and cytokine production pathways in CD4+ T cells is regulated by the antigen-presenting cell. *J Immunol*. 1993; 150(9):3832-3842. (Clone-specific: Activation, Apoptosis)

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