## Technical Data Sheet

# BV605 Hamster Anti-Mouse CD3e

#### **Product Information**

**Material Number:** 563004

Alternate Name: CD3; CD3 epsilon; Cd3e; CD3e; T3e

Size 50 µg 0.2 mg/mlConcentration: 145-2C11 Clone:

H-2Kb specific cytotoxic T lymphocyte clone BM10-37 Immunogen:

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.

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant<sup>TM</sup> Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794).

### 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<sup>TM</sup> BV605 under optimum conditions, and unconjugated antibody and free BD Horizon<sup>TM</sup> BV605 were removed.

## **Application Notes**

#### Application

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Flow cytometry	Routinely Tested	

# **Suggested Companion Products**

Catalog Number	Name Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
563009	BV605 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
563794	Brilliant Stain Buffer	5 mL	(none)

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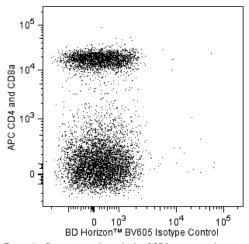
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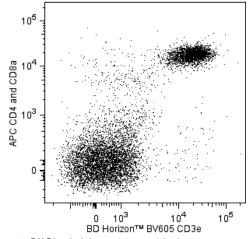
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Two-color flow cytometric analysis of CD3e expressed on mouse splenocytes. BALB/c splenic leucocytes were stained with APC Rat Anti-Mouse CD4 (Cat. No. 553051/561091) and APC Rat Anti-Mouse CD8a (Cat. No. 553035/561093) antibodies and either BD Horizon™ BV605 Armenian Hamster IgG1, κ Isotype Control (Cat. No. 563009; Left Panel) or BD Horizon¹ BV605 Hamster Anti-Mouse CD3e antibody (Cat. No. 563004; 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.

#### **Product Notices**

- 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 for technical protocols.
- 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.
- 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.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 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 BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon™ BV605 conjugate.
- 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.
- 10. CF<sup>TM</sup> is a trademark of Biotium, Inc.

### References

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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: Bioassay, Cytotoxicity, Functional assay, 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-/- mice in the absence of TCR beta chain expression. *Int Immunol.* 1994; 6(7):995-1001. (Biology)

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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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