## Technical Data Sheet

# BV605 Mouse Anti-Human CD3

#### **Product Information**

**Material Number:** 562994

Alternate Name: CD3E; CD3-epsilon; T3E; TCRE

50 Tests Size Vol. per Test: 5 μl SP34-2 Clone:

Purified Human CD3E Protein Immunogen: Isotype: Mouse (BALB/c) IgG1, λ

Reactivity: QC Testing: Rhesus, Cynomolgus, Baboon

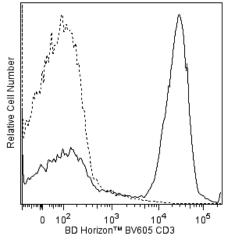
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

### Description

Clone SP34-2 is a mouse IgG1 isotype monoclonal antibody, descendant of SP34 (mouse IgG3), with the same specificity and reactivity pattern as the parent clone. It cross-reacts with a major subset of peripheral blood lymphocytes, but not monocytes or granulocytes, of baboon, and rhesus, cynomolgus, and pigtail macaque monkeys. The distribution on lymphocytes is similar to that observed with normal human donor lymphocytes with the majority of CD3-positive cells being negative when dual stained with antibodies to B or NK cells markers. SP34-2 is also capable of inducing cell proliferation on both human and non-human primate PBMC.

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).



Flow cytometric analysis of CD3 expression on Rhesus macaque peripheral blood lymphocytes. Rhesus macaque whole blood was stained with either a BD Horizon™ BV605 Mouse IgG1, κ Isotype Control (Cat. No. 562652: dashed line histogram) or with BD Horizon™ BV605 Mouse Anti-Human CD3 antibody (Cat. No. 562994; solid line histogram). Erythrocytes were lysed with BD Pharm Lvse™ Lvsing Buffer (Cat. No. 555899). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD  $^{\mathrm{TM}}$  LSR II Flow Cytometer 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 BD Horizon™ BV605 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV605 were removed.

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### **Application Notes**

### Application

Flow cytometry	Routinely Tested	

### **Suggested Companion Products**

Catalog Number	Name	Size	Clone	
554656	Stain Buffer (FBS)	500 mL	(none)	
562652	BV605 Mouse IgG1, κ Isotype Control	50 μg	X40	
555899	Lysing Buffer	100 mL	(none)	
563794	Brilliant Stain Buffer	5 mL	(none)	

#### **Product Notices**

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10<sup>6</sup> cells in a 100-µl experimental
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- An isotype control should be used at the same concentration as the antibody of interest.
- 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.
- CFTM is a trademark of Biotium, Inc.

# References

Bleavins MR, Brott DA, Alvey JD, de la Iglesia FA. Flow cytometric characterization of lymphocyte subpopulations in the cynomolgus monkey (Macaca fascicularis). Vet Immunol Immunopathol. 1993; 37(1):1-13. (Biology)

Blumberg RS, Ley S, Sancho J, Lonberg N, Lacy E, McDermott F, Schad V, Greenstein JL, Terhorst C. Structure of the T-cell antigen receptor: evidence for two CD3 epsilon subunits in the T-cell receptor-CD3 complex. Proc Natl Acad Sci U S A. 1990; 87(18):7220-7224. (Clone-specific: Immunoprecipitation, Western blot) Conrad ML, Davis WC, Koop BF. TCR and CD3 antibody cross-reactivity in 44 species. Cytometry A. 2007; 71(11):925-933. (Biology)

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Jacobsen CN, Aasted B, Broe MK, Petersen JL. Reactivities of 20 anti-human monoclonal antibodies with leucocytes from ten different animal species. Vet Immunol Immunopathol. 1993; 39(4):461-466. (Biology)

Pessano S, Oettgen H, Bhan AK, Terhorst C. The T3/T cell receptor complex: antigenic distinction between the two 20-kd T3 (T3-delta and T3-epsilon) subunits. EMBO J. 1985; 4(2):337-344. (Immunogen: Activation, Dot Blot, Functional assay, Immunoprecipitation, Western blot)

Schlossman SF, Boumsell L, Gilks W, et al, ed. Leukocyte Typing V: White Cell Differentiation Antigens. Oxford: Oxford University Press; 1995. (Clone-specific)

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562994 Rev. 2 Page 2 of 2