# **Technical Data Sheet**

# **BV650 Mouse Anti-Human CD8**

#### **Product Information**

Material Number: 563822

Alternate Name: CD8α; CD8A; CD8 alpha; Leu2; MAL; T8; p32

Tested in Development: Rhesus, Cynomolgus, Baboon

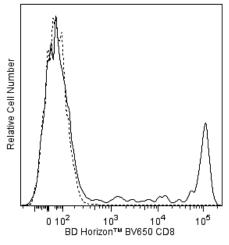
Workshop: IV T171; V T-CD08.03; VI 6T-CD8.1, 6T-081

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

## Description

The RPA-T8 monoclonal antibody specifically binds to CD8 alpha (CD8 $\alpha$ ). CD8 $\alpha$  is a type I transmembrane glycoprotein and a member of the immunoglobulin superfamily. CD8 $\alpha$  is expressed by the majority of thymocytes, by subpopulations of  $\alpha\beta$  T cells and  $\gamma\delta$  T cells and by some NK cells. Cell surface CD8 $\alpha$  is expressed either as a disulfide-linked homodimer (CD8 $\alpha$ ) or as a heterodimer (CD8 $\alpha$ ) when disulfide-bonded to a CD8 beta chain (CD8 $\beta$ ). CD8-positive  $\alpha\beta$  T cells coexpress both CD8 $\alpha$  homodimers and CD8 $\alpha$  heterodimers whereas some  $\gamma\delta$  T cells and NK cells express CD8 $\alpha$  homodimers. CD8 plays important roles in T cell activation and selection. The extracellular IgSF domain of CD8 $\alpha$  binds to a non-polymorphic determinant on HLA class I molecules ( $\alpha$ 3 domain) and enables CD8 to function as a co-receptor with MHC class I-restricted TCR during T cell recognition of antigen. The cytoplasmic domain of CD8 $\alpha$  associates with Lck, a Src family protein tyrosine kinase that is involved in intracellular signaling. The RPA-T8 and HIT8a monoclonal antibodies are not cross-blocking.

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.



Flow cytometric analysis of CD8α expression on human peripheral blood lymphocytes. Human whole blood was stained with either BD Horizon™ BV650 Mouse Anti-Human CD8 antibody (Cat. No. 563821/563822; solid line histogram) or BD Horizon™ BV650 Mouse lgG1, κ Isotype Control (Cat. No. 563231; dashed line histogram). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ 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<sup>TM</sup> BV650 under optimum conditions, and unconjugated antibody and free BD Horizon<sup>TM</sup> BV650 were removed.

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#### **Application Notes**

# Application

Flow cytometry	
Flow cytometry	Routinely Tested

# **Suggested Companion Products**

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563231	BV650 Mouse IgG1, k Isotype Control	50 μg	X40
563821	BV650 Mouse Anti-Human CD8	100 tests	RPA-T8
349202	BD FACS <sup>TM</sup> Lysing Solution	100 ml	(none)
555899	Lysing Buffer	100 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 sample (a test).
- 2. An isotype control should be used at the same concentration as the antibody of interest.
- 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
- 6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- 7. Brilliant Violet<sup>TM</sup> 650 is a trademark of Sirigen.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV*. New York, NY: Oxford University Press; 1989:1-1182. (Clone-specific) Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens*. New York: Oxford University Press; 1995. (Clone-specific)

Zola H, Swart B, Nicholson I, Voss E. Leukocyte and Stromal Cell Molecules. The CD Markers. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2007:1-581. (Biology)

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