

## Technical Data Sheet

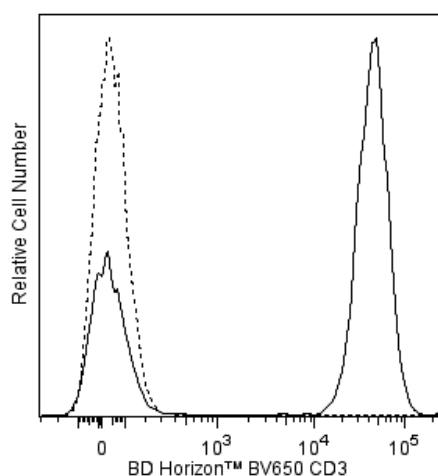
**BV650 Mouse Anti-Human CD3****Product Information**

<b>Material Number:</b>	<b>563852</b>
<b>Alternate Name:</b>	CD3ε; CD3E; T3E; TCRE; T-cell surface antigen T3/Leu-4 epsilon
<b>Size:</b>	100 tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	UCHT1
<b>Immunogen:</b>	Human infant thymocytes and peripheral blood lymphocytes from a Sézary Syndrome donor
<b>Isotype:</b>	Mouse (BALB/c) IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Workshop:</b>	III 471
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**

The UCHT1 monoclonal antibody specifically binds to the human CD3ε-chain, a 20-kDa subunit of the CD3/T cell antigen receptor complex. CD3ε is expressed on 70-80% of normal human peripheral blood lymphocytes and 60-85% of thymocytes. Studies from the HLDA Workshop show that this antibody is mitogenic for CD3ε-positive cells when used in conjunction with costimulatory agents such as pokeweed mitogen or anti-CD28 antibody. CD3 plays a central role in signal transduction during antigen recognition. The UCHT1 antibody stains both surface and intracellular CD3ε unlike the other CD3 clone, HIT3a, that stains only extracellular CD3ε.

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 CD3 expression on human peripheral blood lymphocytes.** Human whole blood was stained with the BD Horizon™ BV650 Mouse Anti-Human CD3 antibody (Cat. No. 563851/563852; solid line histogram) or with BD Horizon™ BV650 Mouse IgG1, 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 Cytometry 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™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

**Application Notes****Application**

Flow cytometry

Routinely Tested

**BD Biosciences**

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## 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
563851	BV650 Mouse Anti-Human CD3	25 tests	UCHT1
349202	BD FACS™ Lysing Solution	100 ml	(none)
555899	Lysing Buffer	100 ml	(none)

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-µl experimental sample (a test).
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/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. 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).
8. Brilliant Violet™ 650 is a trademark of Sirigen.

## References

Beverley PC, Callard RE. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur J Immunol.* 1981; 11(4):329-334. (Immunogen: Flow cytometry, Fluorescence activated cell sorting)

Burns GF, Boyd AW, Beverley PC. Two monoclonal anti-human T lymphocyte antibodies have similar biologic effects and recognize the same cell surface antigen. *J Immunol.* 1982; 129(4):1451-1457. (Clone-specific: Blocking, Functional assay, Immunoprecipitation, Inhibition, Radioimmunoassay)

Ernst DN, Shih CC. CD3 complex. *J Biol Regul Homeost Agents.* 2000; 14(3):226-229. (Biology)

Knapp W, Dörken B, Gilks WR, et al, ed. *Leukocyte Typing IV*. New York, NY: Oxford University Press; 1989:1-1182. (Clone-specific: Flow cytometry)

McMichael AJ, Beverley PCL, Gilks W, et al, ed. *Leukocyte Typing III: White Cell Differentiation Antigens*. New York: Oxford University Press; 1987. (Clone-specific: Flow cytometry)

Van Wauwe JP, Goossens JG, Beverley PC. Human T lymphocyte activation by monoclonal antibodies; OKT3, but not UCHT1, triggers mitogenesis via an interleukin 2-dependent mechanism. *J Immunol.* 1984; 133(1):129-132. (Clone-specific: Flow cytometry, Functional assay, Stimulation)

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