

Technical Data Sheet

V500 Mouse Anti-Human CD3

Product Information

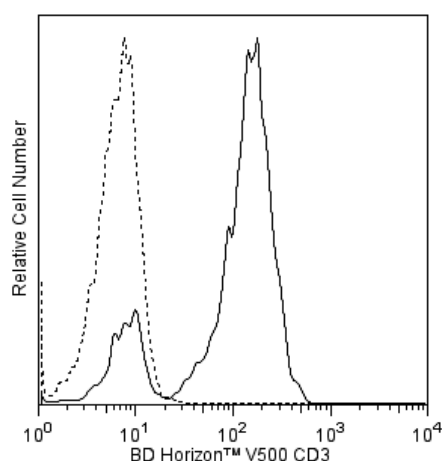
Material Number:	561416
Alternate Name:	CD3ε; CD3E; T3E; TCRE ; T-cell surface antigen T3/Leu-4 epsilon
Size:	100 tests
Vol. per Test:	5 µl
Clone:	UCHT1
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III 471
Storage Buffer:	Aqueous buffered solution containing protein stabilizer, glycerol 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 is conjugated to BD Horizon™ V500, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser with an Ex max of 415 nm and Em Max at 500 nm. BD Horizon V500 conjugates emit at a similar wavelength to AmCyan yet exhibit reduced spillover into the FITC channel. For more information on BD Horizon V500, visit bdbiosciences.com/colors.

When compensating dyes in this spectral range (such as Horizon™ V500 and AmCyan), the most accurate compensation can be obtained using single stained cellular controls. Due to spectral differences between cells and beads in this channel, using BD CompBeads can result in spillover errors for V500 and AmCyan reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different V500 reagents (e.g., CD4 vs. CD45) can have slightly different fluorescence spillover levels. Therefore, it may also be necessary to use clone specific compensation controls when using these reagents.



Flow cytometric analysis of CD3 expressed on human peripheral lymphocytes. Human whole blood was stained with the BD Horizon™ V500 Mouse anti-Human CD3 antibody (Cat. No. 561416/561417; solid line histogram) or with a BD Horizon™ V500 Mouse IgG1, κ Isotype Control (Cat. No. 560787; dashed line histogram). The erythrocytes were lysed with BD PharmLyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ V500 under optimum conditions, and unreacted BD Horizon™ V500 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
555899	Lysing Buffer	100 ml	(none)
560787	V500 Mouse IgG1, κ Isotype Control	0.1 mg	X40
561417	V500 Mouse Anti-Human CD3	25 tests	UCHT1
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. BD Horizon™ V500 has a maximum absorption of 415 nm and maximum emission of 500 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

References

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