Technical Data Sheet

BUV737 Mouse Anti-Human CD3

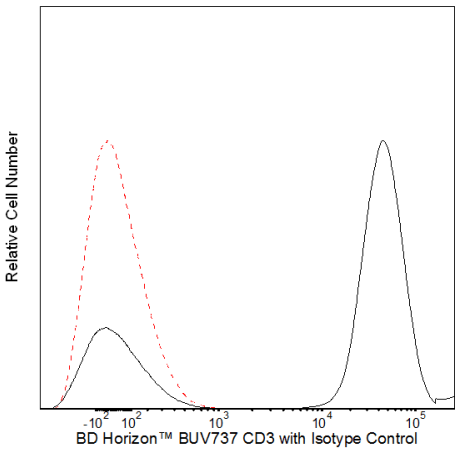
Product Information

Material Number:	**564307**
Alternate Name:	CD3e; CD3E; T3E; TCRE; T-cell surface antigen T3/Leu-4 epsilon
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	UCHT1
Immunogen:	Human infant thymocytes and peripheral blood lymphocytes from a Sézary Syndrome donor
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III 471
Storage Buffer:	Aqueous buffered solution containing ≤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 BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Flow cytometric analysis of CD3 expression on human peripheral blood lymphocytes. *Human whole blood was stained with either BD Horizon™ BUV737 Mouse IgG1, κ Isotype Control (Cat. No. 564299; dashed line histogram) or BD Horizon BUV737 Mouse Anti-Human CD3 antibody (Cat. No. 564307/564308; solid 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 BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

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. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554657	Stain Buffer (BSA)	500 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
564299	BUV737 Mouse IgG1, κ Isotype Control	50 µg	X40
564308	BUV737 Mouse Anti-Human CD3	25 Tests	UCHT1
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
8. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

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. W. Knapp .. et al., ed. *Leucocyte typing IV : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1989:1-1182. (Clone-specific: Flow cytometry)

McMichael AJ. A.J. McMichael .. et al., ed. *Leucocyte typing III : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1987:1-1050. (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)