Technical Data Sheet

BUV737 Rat Anti-Mouse CD3 Molecular Complex

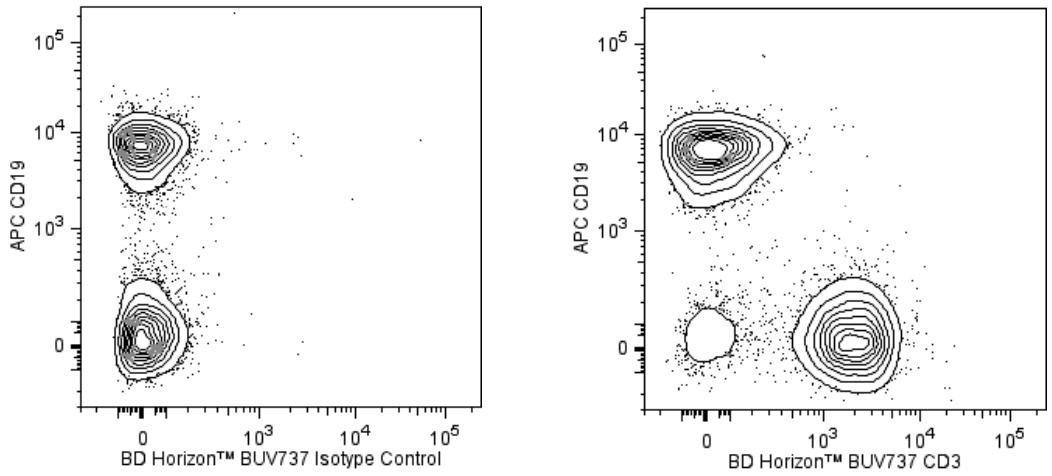
Product Information

Material Number:	**564380**
Alternate Name:	CD3; CD3 epsilon; Cd3e; CD3ε; T3e
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	17A2
Immunogen:	γδ TCR-positive T-T hybridoma D1
Isotype:	Rat (SD) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 17A2 monoclonal antibody specifically binds to the T-cell receptor-associated CD3 complex that is expressed on many thymocytes and mature T lymphocytes. Plate-bound 17A2 antibody has been reported to induce IL-2 production by cultured T cells in the absence of accessory cells. The binding of the 17A2 antibody to T cells can be blocked by the anti-CD3e mAb 145-2C11 (Cat. No. 557306/553058/550275). This suggests that the 17A2 antibody recognizes an epitope of the CD3 epsilon chain. In vivo treatment with 17A2 antibody has been reported to partially deplete T lymphocytes and temporarily down-modulates CD3 expression on T cells.

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.



Two-color flow cytometric analysis of CD3 Molecular Complex expression on mouse splenocytes. *Splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with APC Rat Anti-Mouse CD19 antibody (Cat. No. 550992/561738) and either BD Horizon™ BUV737 Rat IgG2b, κ Isotype Control (Cat. No. 564295; Left Panel) or BD Horizon BUV737 Rat Anti-Mouse CD3 Molecular Complex antibody (Cat. No. 564380; Right Panel). Two-color flow cytometric contour plots showing the correlated expression of CD3 (or Ig Isotype control staining) versus CD19 were derived from gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. 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 BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

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Application Notes

Application

Flow cytometry	Routinely Tested

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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564295	BUV737 Rat IgG2b, κ Isotype Control	50 µg	R35-38
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
563794	Brilliant Stain Buffer	100 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
550992	APC Rat Anti-Mouse CD19	0.1 mg	1D3
561738	APC Rat Anti-Mouse CD19	25 µg	1D3
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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/pharmingen/protocols for technical protocols.

References

Miescher GC, Schreyer M, MacDonald HR. Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. *Immunol Lett.* 1989; 23(2):113-118. (Immunogen: Cytotoxicity, Flow cytometry, Functional assay, Immunohistochemistry, Immunoprecipitation, Stimulation)

Mysliwietz J, Thierfelder S. Antilymphocytic antibodies and marrow transplantation. XII. Suppression of graft-versus-host disease by T-cell-modulating and depleting antimouse CD3 antibody is most effective when preinjected in the marrow recipient. *Blood.* 1992; 80(10):2661-2667. (Clone-specific: Depletion, Flow cytometry, Functional assay, In vivo exacerbation)

Wu L, Antica M, Johnson GR, Scollay R, Shortman K. Developmental potential of the earliest precursor cells from the adult mouse thymus. *J Exp Med.* 1991; 174(6):1617-1627. (Clone-specific: Cell separation, Depletion)