

Technical Data Sheet

BUV615 Rat Anti-Mouse CD8a

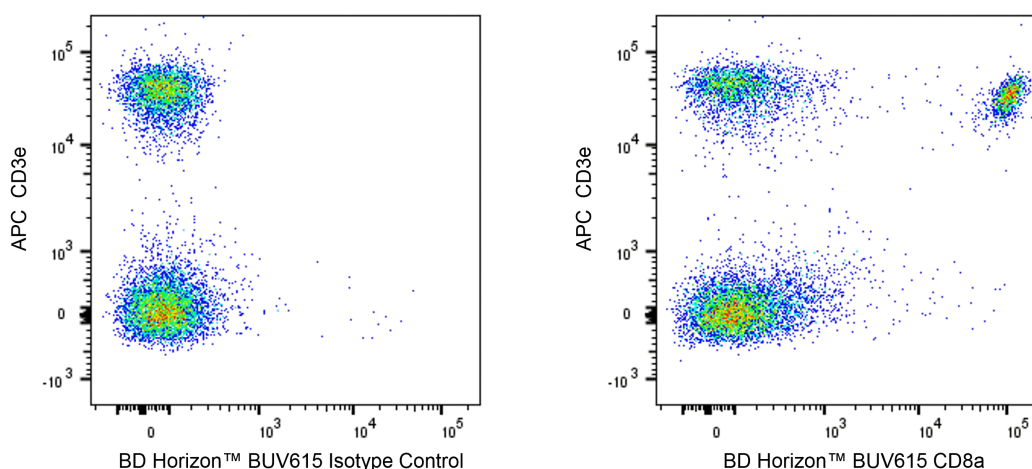
Product Information

Material Number:	613004
Alternate Name:	CD8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	53-6.7
Immunogen:	Mouse Spleen Cells or Thymocyte Membranes
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 53-6.7 monoclonal antibody specifically binds to the 38 kDa α and 34 kDa α' chains of the CD8 differentiation antigen (Ly-2 or Lyt-2) of all mouse strains tested. The CD8 α and α' chains (CD8a) form heterodimers with the CD8 β chain (CD8b, Ly-3, or Lyt-3) on the surface of most thymocytes. A subpopulation of mature T lymphocytes (i.e., MHC class I-restricted T cells, including most T suppressor/cytotoxic cells) expresses almost exclusively the CD8 $\alpha\beta$ heterodimer. Subsets of $\gamma\delta$ TCR-bearing T cells, intestinal intrapithelial lymphocytes, and dendritic cells express CD8a without CD8b. It has been suggested that the expression of the CD8a/CD8b heterodimer is restricted to T lymphocytes which matured in the thymus or in an extrathymic environment that had been influenced by thymus-initiated neuroendocrine signals. CD8 is an antigen coreceptor on the T-cell surface which interacts with MHC class I molecules on antigen-presenting cells or epithelial cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase lck (p56 [lck]). The CD8 α and α' chains arise from alternatively spliced messengers of a single *CD8a* gene. The longer α form associates with p56 [lck] via a CXCP motif in its cytoplasmic domain, which it shares with CD4, but not with CD8b. The truncated α' chain is unable to associate with p56 [lck], and it may function to attenuate the CD8-mediated costimulatory signal during intrathymic T-cell maturation. In vivo and in vitro treatment with 53-6.7 mAb has reportedly been effective at depleting CD8⁺ peripheral T lymphocytes. The 53-6.7 antibody has also been reported to cross-react with CD8 α - and α' -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.

The antibody was conjugated to BD Horizon BUV615 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 615 nm. BD Horizon Brilliant BUV615 can be excited by the ultraviolet laser (355 nm) and detected with a 610/20 filter and a 595 nm LP. Due to the excitation of the acceptor dye by the blue/yellow-green laser line, there may be significant spillover into channels detecting PE-CF594 like emissions (eg, 610/20-nm filter).



Two-color flow cytometric analysis of CD8a expression on mouse splenocytes. Mouse 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 Hamster Anti-Mouse CD3e antibody (Cat. No. 553066/561826) and either BD Horizon™ BUV615 Rat IgG2a, κ Isotype Control (Cat. No. 613005; Left Plot) or BD Horizon BUV615 Rat Anti-Mouse CD8a antibody (Cat. No. 613004; Right Plot) at 0.5 µg/test. BD Via-Probe™ Cell Viability 7-AAD Solution (Cat. No. 555815/555816) was added to cells right before analysis. The bivariate pseudocolor density plot showing the correlated expression of CD8a (or Ig Isotype control staining) versus CD3e was derived from gated events with the forward and side light-scatter characteristic of viable (7-AAD negative) splenic leucocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software. Data shown on this Technical Data Sheet are not lot specific.

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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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

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)
613005	BUV615 Rat IgG2a, κ Isotype Control	50 µg	R35-95
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. 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 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.
6. BD Horizon Brilliant Ultraviolet 615 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.
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

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