

## Technical Data Sheet

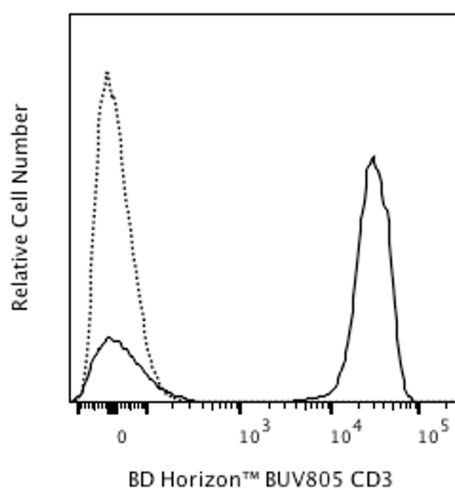
**BUV805 Mouse Anti-Human CD3****Product Information**

<b>Material Number:</b>	<b>612894</b>
<b>Alternate Name:</b>	CD3-epsilon; CD3E; Leu4; T-cell surface antigen T3/Leu-4 epsilon chain; T3E
<b>Size:</b>	25 Tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	SK7 (also known as Leu-4)
<b>Immunogen:</b>	Human Thymocytes
<b>Isotype:</b>	Mouse (BALB/c) IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Workshop:</b>	II T118; III T492
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The SK7 (Leu-4) monoclonal antibody specifically binds to the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex. This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kDa. The antigen recognized by CD3 antibodies is noncovalently associated with either  $\alpha/\beta$  or  $\gamma/\delta$  TCR (70 to 90 kDa). The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes 60% to 85% of thymocytes and on Purkinje cells in the cerebellum. The soluble form of this antibody has a mitogenic effect on most peripheral blood T lymphocytes, provided appropriate functional monocytes are present.

The antibody was conjugated to BD Horizon BUV805 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 805 nm. BD Horizon Brilliant BUV805 can be excited by the ultraviolet laser (355 nm) and detected with a 820/60 nm filter and a 770 nm LP.



**Flow cytometric analysis of CD3 expression on human peripheral blood lymphocytes.** Human whole blood was stained with either BD Horizon™ BUV805 Mouse IgG1, κ Isotype Control (Cat. No. 612897; dashed line histogram) or BD Horizon BUV805 Mouse Anti-Human CD3 antibody (Cat. No. 612893/612894; solid line histogram). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The fluorescence histogram showing CD3 expression (or Ig Isotype control staining) was derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software.

**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 BUV805 under optimum conditions, and unconjugated antibody and free BD Horizon BUV805 were removed.

**Application Notes****Application**

Flow cytometry

Routinely Tested

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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 CompBeads. This will 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
612893	BUV805 Mouse Anti-Human CD3	100 Tests	SK7
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
349202	BD FACSTM Lysing Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
612897	BUV805 Mouse IgG1, k Isotype Control	50 µg	X40

### 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. 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 [wwwbdbiosciences.com/colors](http://wwwbdbiosciences.com/colors).
5. BD Horizon Brilliant Ultraviolet 805 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 [wwwbdbiosciences.com/pharmingen/protocols](http://wwwbdbiosciences.com/pharmingen/protocols) for technical protocols.

### References

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