Technical Data Sheet

BUV737 Mouse Anti-Human CD25

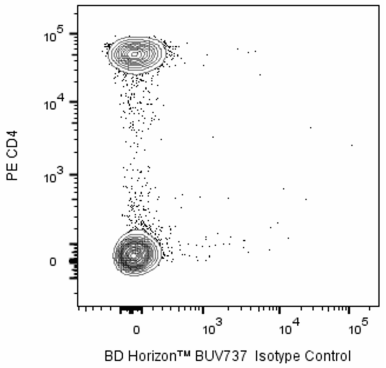
Product Information

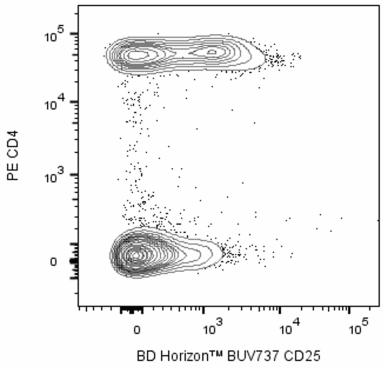
Material Number:	**612806**
Alternate Name:	IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	2A3
Immunogen:	Human Phytohemagglutinin-activated T Cells
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III A769,T153; IV A8
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

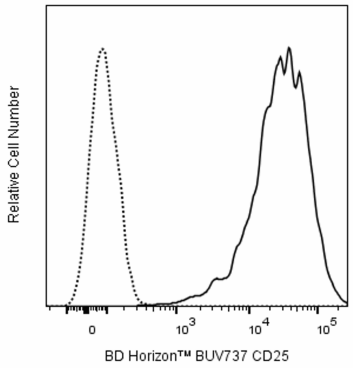
The 2A3 monoclonal antibody specifically binds to human CD25, the low-affinity alpha subunit of the Interleukin-2 Receptor (IL- 2Rα). CD25 associates with CD122 (IL-2Rβ chain) and CD132 (common γ chain or γc) to form the high-affinity signal-transducing IL-2R complex. CD25 is expressed by subsets of thymocytes and peripheral blood lymphocytes including CD4+CD25+ regulatory T cells and memory T cells. CD25 antigen density increases on activated T cells including phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes. High levels of CD25 can be expressed by T lymphocytes from mixed lymphocyte cultures and by human T-lymphocyte leukemia virus (HTLV)-infected T-lymphocyte leukemia lines, for example, HUT-102. CD25 can also be expressed by activated B cells and macrophages. Recombinant IL-2 blocks the binding of the 2A3 antibody to PHA-activated T lymphocytes.

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 CD25 expression on unstimulated peripheral blood lymphocytes. *Human whole blood was stained with PE Mouse Anti-Human CD4 antibody (555347/561843/561844) and either BD Horizon™ BUV737 Mouse IgG1, κ Isotype Control (Cat. No. 612758; Left Plot) or BD Horizon BUV737 Mouse Anti-Human CD25 antibody (Cat. No. 612806/612807; Right Plot). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). The two-color flow cytometric contour plot showing the correlated expression of CD25 (or Ig Isotype control staining) versus CD4 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™ Cell Analyzer System and FlowJo™ software.*



Flow cytometric analysis of CD25 expression on activated peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were stimulated for 3 days with Phytohemagglutinin. The cells were stained with either BD Horizon BUV737 Mouse IgG1, κ Isotype Control (dashed line histogram) or BD Horizon BUV737 Mouse Anti-Human CD25 antibody (solid line histogram). The fluorescence histogram showing CD25 expression (or Ig Isotype control staining) was derived from gated events with the forward and side light-scatter characteristics of viable lymphoblasts. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ software.*

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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 with BD Horizon BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 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 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
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
612807	BUV737 Mouse Anti-Human CD25	25 Tests	2A3
612758	BUV737 Mouse IgG1, κ Isotype Control	50 µg	X40
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(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 www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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