**Technical Data Sheet**

**BUV395 Rat Anti-Mouse CD25**

**Product Information**

- **Material Number:** 564022
- **Alternate Name:** Interleukin-2 receptor alpha chain; IL-2Ra; IL-2R α; Il2ra; IL-2R p55
- **Size:** 50 µg
- **Concentration:** 0.2 mg/ml
- **Clone:** PC61
- **Immunogen:** IL-2-dependent cytolytic mouse T-cell clone B6.1
- **Isotype:** Rat (OFA) IgG1, λ
- **Reactivity:** QC Testing: Mouse
- **Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**

The PC61 monoclonal antibody specifically binds to CD25, the low-affinity IL-2 Receptor α chain (IL-2R α, p55) expressed on activated T and B lymphocytes from all mouse strains tested. IL-2Rα by itself is not a signaling receptor. However, it can combine with IL-2 Receptor β (CD122) and γc (CD132) chains to form high-affinity, signaling receptor complexes for IL-2. Resting T and B lymphocytes and resting and activated NK cells do not express IL-2Rα. CD25 is transiently expressed at a low level during normal B-cell development in the bone marrow on the CD45R/B220low TdT- slg- Pre-B/Pre-B-II and CD45R/B220low TdT- slgM+ slgD- immature B stages, but not on the CD45R/B220low TdT- slg- Pro-B/Pre-B-I stage nor on CD45R/B220high TdT- slgM+ slgD+ mature B cells. It is expressed at a higher level during a very early stage of T-cell development in fetal and adult thymus. Peripheral CD25+CD4+ lymphocytes called regulatory T (Treg) cells are involved in the maintenance of self-tolerance. It has also been reported that dendritic cells express CD25, recognized by mAb 7D4. The PC61 antibody recognizes an epitope of CD25 which is distinct from the IL-2 binding site and from those recognized by mAbs 3C7 and 7D4. It blocks binding of IL-2 to CD25, presumably by inducing a conformational change in CD25.

The antibody was conjugated to BD Horizon™ BUV395 which has been exclusively developed by BD Biosciences as an optimal dye for use on a 355 nm laser equipped instrument. With an Ex Max at 348 nm and an Em Max at 395 nm, this dye has virtually no spillover into any other detector. BD Horizon BUV395 can be excited with a 355 nm laser and detected with a 379/28 filter.

**Flow cytometric analysis of CD25 expression on unstimulated and stimulated mouse splenocytes.**

- **Left and Middle Panels:** 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 FITC Rat Anti-Mouse CD4 antibody (Cat. No. 553047/553046/561835) and either BD Horizon™ BUV395 Rat IgG1, λ Isotype Control (Cat. No. 564015; Left Panel) or BD Horizon™ BUV395 Rat Anti-Mouse CD25 antibody (Cat. No. 564022; Middle Panel). Two-color flow cytometric dot plots show the correlated expression patterns for CD25 (or Ig Isotype control staining) versus CD4 for events with the forward and side light-scatter characteristics of viable splenocytes.

- **Right Panel:** Mouse splenic leucocytes were stimulated for 3 days with concanavalin A. The cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody and then stained with either BD Horizon™ BUV395 Rat IgG1, λ Isotype Control (dashed line histogram) or BD Horizon™ BUV395 Rat Anti-Mouse CD25 antibody (solid line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphoblasts.

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™ BUV395 under optimum conditions, and unconjugated antibody and free BD Horizon™ BUV395 were removed.

Application Notes

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<thead>
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<th>Application</th>
<th>Routinely Tested</th>
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<tbody>
<tr>
<td>Flow cytometry</td>
<td>Routinely Tested</td>
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Recommended Assay Procedure:
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).

Suggested Companion Products

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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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. BD Horizon Brilliant Ultraviolet 395 is covered by one or more of the following US patents: 8,158,444; 8,575,303; 8,354,239.

References

Chen J, Ma A, Young F, All FW. IL-2 receptor alpha chain expression during early B lymphocyte differentiation. Int Immunol. 1984; 6(8):1265-1268. (Biology)


