Technical Data Sheet

BV421 Rat Anti-Mouse CD25

Product Information

Material Number: 564370
Alternate Name: Interleukin-2 receptor alpha chain; IL-2RA; IL-2Rα; Il2ra; IL-2R p55
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: 3C7
Immunogen: IL-2-dependent BALB/c mouse cell line
Isotype: Rat (LEW) IgG2b, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 3C7 monoclonal antibody specifically binds to CD25, the low affinity IL-2 Receptor (IL-2Rα, 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-I and CD45R/B220low TdT- slgM+ slgD- immature B stages, but not on the CD45R/B220low TdT+ slg- Pro-B/Pro B-I stage nor on CD45R/B220high TdTslgM+ 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+ T 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, another CD25-specific antibody. The 3C7 antibody recognizes an epitope of CD25 which is distinct from those recognized by other CD25-specific mAbs, 7D4 and PC61. 3C7 blocks the binding of IL-2 to CD25.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue conjugates.

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

Left and Middle Panels: Freshly prepared 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 Rat Anti-Mouse CD4 antibody (Cat. No. 553051/561091) and either BD Horizon™ BV421 Rat IgG2b, κ Isotype Control (Cat. No. 562603; Left Panel) or BD Horizon BV421 Rat Anti-Mouse CD25 antibody (Cat. No. 564370; Middle Panel). Two-color flow cytometric contour plots showing the correlated expression patterns for CD25 (or Ig Isotype Control staining) versus CD4 were generated for gated events with the forward and side light-scatter characteristics of viable lymphocytes.

Right Panel: Mouse splenic leucocytes were stimulated with concanavalin A for 3 days. The cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™). The cells were then stained with either BD Horizon BV421 Rat IgG2b, κ Isotype Control (dashed line histogram) or BD Horizon BV421 Rat Anti-Mouse CD25 antibody (solid line histogram). The fluorescence histograms were derived from gated 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.

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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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™
BV421 were removed.

Application Notes

Application

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<th>Application</th>
<th>Routinely Tested</th>
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<tr>
<td>Flow cytometry</td>
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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. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References


Malek TR, Robb RJ, Shevach EM. Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor-ligand complex. *Proc Natl Acad Sci U S A.* 1983; 80(18):5694-5698. (Biology)


