

Technical Data Sheet

PerCP-Cy™ 5.5 Rat Anti-Mouse CD25

Product Information

Material Number:	561112
Alternate Name:	Interleukin-2 receptor alpha chain; IL-2RA; IL-2R α ; IL2ra; IL-2R p55
Size:	25 μ g
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 $\leq 0.09\%$ sodium azide.

Description

The PC61 antibody reacts with 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-2R α by itself is not a signaling receptor. However, it can combine with IL-2 Receptor β (CD122) and γ c (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-2R α . CD25 is transiently expressed at a low level during normal B-cell development in the bone marrow on the CD45R/B220low TdT- sIg- Pre-B/Pre-B-II and CD45R/B220low TdT- sIgM+ sIgD- immature B stages, but not on the CD45R/B220low TdT+ sIg- Pro-B/Pre-B-I stage nor on CD45R/B220high TdT- sIgM+ sIgD+ 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 (Cat. No. 553068). The PC61 antibody recognizes an epitope of CD25 which is distinct from the IL-2 binding site and from those recognized by mAbs 3C7 (Cat. No. 557364) and 7D4 (Cat. No. 553068). It blocks binding of IL-2 to CD25, presumably by inducing a conformational change in CD25.

Use of this product can fall under one or more claims of the following patents licensed to Becton, Dickinson and Company: US Patent Nos. 5,445,939, 5,656,446, 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australian Patent No. 615,880; and Japanese Patent No. 2,769,156.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

For detection of low-density CD25 expression, we recommend the use of the PE conjugate of PC61 antibody (Cat. No. 553866) or the biotin conjugate of the 7D4 antibody (Cat. No. 553069/553070) with a "bright" second-step reagent, such as Streptavidin-PE (Cat. No. 554061). PerCP-Cy5.5 tandem fluorochrome emission is collected in the Fluorescence-3 (FL3) channel of BD FACScan™ and BD FACSCalibur™ Flow Cytometry Systems. PerCP-Cy5.5-labelled antibodies can be used with FITC- and R-PE-labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For tandem conjugates incorporating PerCP (e.g., PerCP-Cy5.5), the excitation and emission properties of PerCP and the kinetics of energy exchange between the fluorochromes of the tandem dye may limit their effectiveness on high-speed and/or sorting flow cytometers. Therefore, for third-color flow-cytometric analysis using ≥ 25 -mW laser power, we recommend PE-Cy7-conjugated reagents (e.g., Cat. No. 552880).

Suggested Companion Products

Catalog Number	Name	Size	Clone
551072	PerCP-Cy™ 5.5 Rat IgG1, λ Isotype Control	0.1 mg	A110-1

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

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6. PerCP is a photosynthetic accessory pigment from *Glenodinium* species of dinoflagellates, which is excited by the 488-nm light of an Argon ion laser and fluoresces at 675 nm. Therefore, PerCP-labelled antibodies can be used with FITC- and R-PE-labelled reagents in most single-laser flow cytometers with no significant spectral overlap of PerCP fluorescence with that of FITC or R-PE. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For third-color flow-cytometric analysis using ≥ 25 -mW laser power, we recommend PE-Cy5-, PE-Cy7-, or PerCP-Cy5.5-conjugated reagents.
7. PerCP-Cy5.5-labelled antibodies can be used with FITC- and R-PE-labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
8. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
9. 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.

References

- Ceredig R, Lowenthal JW, Nabholz M, MacDonald HR. Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells. *Nature*. 1985; 314(6006):98-100. (Clone-specific: Blocking, Immunohistochemistry)
- Chen J, Ma A, Young F, Alt FW. IL-2 receptor alpha chain expression during early B lymphocyte differentiation. *Int Immunol*. 1994; 6(8):1265-1268. (Biology)
- Crowley M, Inaba K, Witmer-Pack M, Steinman RM. The cell surface of mouse dendritic cells: FACS analyses of dendritic cells from different tissues including thymus. *Cell Immunol*. 1989; 118(1):108-125. (Biology)
- Garni-Wagner BA, Witte PL, Tutt MM, et al. Natural killer cells in the thymus. Studies in mice with severe combined immune deficiency. *J Immunol*. 1990; 144(3):796-803. (Biology)
- Godfrey DI, Kennedy J, Mombaerts P, Tonegawa S, Zlotnik A. Onset of TCR- β gene rearrangement and role of TCR- β expression during CD3-CD4-CD8-thymocyte differentiation. *J Immunol*. 1994; 152(10):4783-4792. (Biology)
- Greimers R, Trebak M, Moutschen M, Jacobs N, Boniver J. Improved four-color flow cytometry method using fluo-3 and triple immunofluorescence for analysis of intracellular calcium ion ([Ca²⁺]) fluxes among mouse lymph node B- and T-lymphocyte subsets. *Cytometry*. 1996; 23(3):205-217. (Biology)
- Lowenthal JW, Corthesy P, Tougne C, Lees R, MacDonald HR, Nabholz M. High and low affinity IL 2 receptors: analysis by IL 2 dissociation rate and reactivity with monoclonal anti-receptor antibody PC61. *J Immunol*. 1985; 135(6):3988-3994. (Immunogen)
- Lowenthal JW, Zubler RH, Nabholz M, MacDonald HR. Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes. *Nature*. 1985; 315(6021):669-672. (Clone-specific: Blocking, Immunoprecipitation)
- Moreau JL, Nabholz M, Diamantstein T, Malek T, Shevach E, Theze J. Monoclonal antibodies identify three epitope clusters on the mouse p55 subunit of the interleukin 2 receptor: relationship to the interleukin 2-binding site. *Eur J Immunol*. 1987; 17(7):929-935. (Clone-specific: Blocking)
- Pollard AM, Lipscomb MF. Characterization of murine lung dendritic cells: similarities to Langerhans cells and thymic dendritic cells. *J Exp Med*. 1990; 172(1):159-167. (Biology)
- Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med*. 2000; 192(2):295-302. (Biology)
- Rolink A, Grawunder U, Winkler TH, Karasuyama H, Melchers F. IL-2 receptor alpha chain (CD25, TAC) expression defines a crucial stage in pre-B cell development. *Int Immunol*. 1994; 6(8):1257-1264. (Biology)
- Shapiro HM. *Practical Flow Cytometry, 3rd Edition*. New York: Wiley-Liss, Inc; 1995:280-281. (Biology)
- Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med*. 2000; 192(2):303-309. (Biology)
- Taniguchi T, Minami Y. The IL-2/IL-2 receptor system: a current overview. *Cell*. 1993; 73(1):5-8. (Biology)