

Technical Data Sheet

PerCP-Cy™ 5.5 Rat Anti-Mouse CD25

Product Information

Material Number:	551071
Alternate Name:	Interleukin-2 receptor alpha chain; IL-2RA; IL-2R α ; IL2ra; IL-2R p55
Size:	0.1 mg
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 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-2R α by itself is not a signaling receptor. However, it can combine with IL-2 Receptor β (CD122) and γ (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. 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.

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

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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
561112	PerCP-Cy™5.5 Rat Anti-Mouse CD25	25 μ g	PC61

Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 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.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

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4. 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.
5. 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.
6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
7. Cy is a trademark of GE Healthcare.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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