Technical Data Sheet

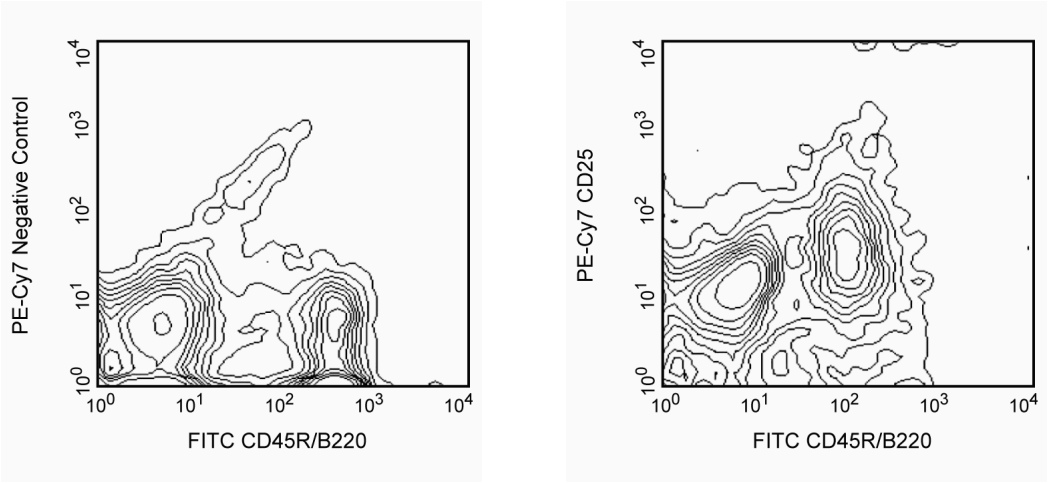
PE-Cy™7 Rat Anti-Mouse CD25

Product Information

Material Number:	**561780**
Alternate Name:	Interleukin-2 receptor alpha chain; IL-2RA; IL-2Rα; Il2ra; IL-2R p55
Size:	25 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 ≤0.09% sodium azide.

Description

The PC61 antibody reacts with CD25, the low-affinity IL-2 Receptor α chain (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 c 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- 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.



Two-color analysis of the expression of CD25 in bone marrow. *BALB/c bone marrow leukocytes were stained with FITC Rat anti-Mouse CD45R/B220 mAb (Cat. No. 553087/553088) and either PE-Cy™7 Rat IgG1, λ Isotype Control mAb (Cat. No. 552869, left panel) or PE-Cy™7 Rat anti-Mouse CD25 mAb (right panel). Please note that the dead leukocytes were not excluded in this experiment, and the typical diagonal dead-cell population appears in the left panel. The same dead-cell population is partially obscured in the right panel. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
553087	FITC Rat Anti-Mouse CD45R/B220	0.1 mg	RA3-6B2
552869	PE-Cy™7 Rat IgG1, λ Isotype Control	0.1 mg	A110-1
554656	Stain Buffer (FBS)	500 ml	(none)

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.
4. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. 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.
6. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
7. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
8. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
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.
10. Use of these products to measure activation antigens expressed on mononuclear cell subsets for the purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: 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.
11. An isotype control should be used at the same concentration as the antibody of interest.

References

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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)

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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)

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