

Technical Data Sheet

APC-R700 Rat Anti-Mouse CD25

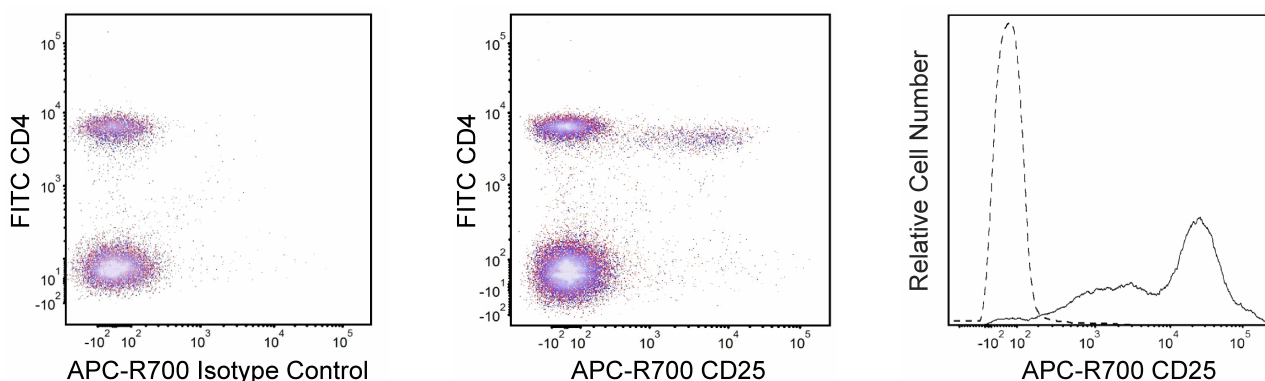
Product Information

Material Number:	565135
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 protein stabilizer and $\leq 0.09\%$ 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 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. 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.

This antibody was conjugated to BD Horizon APC-R700, which has been developed exclusively by BD Biosciences as a better alternative to Alexa Fluor® 700. APC-R700 excites and emits at similar wavelengths to Alexa Fluor® 700 yet exhibits significantly improved brightness. This dye can be excited by the red laser and detected with the same filter set as Alexa Fluor® (eg, 730/45-nm 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. 553141/5 53142). The cells were then stained with FITC Rat Anti-Mouse CD4 antibody (Cat. No. 553047/553046/561835) and either BD Horizon™ APC-R700 Rat IgG1, λ Isotype Control (Cat. No. 565133; Left Panel) or BD Horizon APC-R700 Rat Anti-Mouse CD25 antibody (Cat. No. 565134/565135; Middle Panel). Two-color flow cytometric dot plots showing the correlated expression of CD25 (or Ig Isotype control staining) versus CD4 were derived from gated 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 APC-R700 Rat IgG1, λ Isotype Control (dashed line histogram) or BD Horizon APC-R700 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 LSRFortessa™ Cell Analyzer 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 APC-R700 under optimum conditions, and unconjugated antibody and free BD Horizon APC-R700 were removed.

Application Notes

Application

Flow cytometry

Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
553047	FITC Rat Anti-Mouse CD4	0.5 mg	RM4-5
553046	FITC Rat Anti-Mouse CD4	0.1 mg	RM4-5
561835	FITC Rat Anti-Mouse CD4	25 µg	RM4-5
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
565133	APC-R700 Rat IgG1, λ Isotype Control	0.1 mg	A110-1
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
565134	APC-R700 Rat Anti-Mouse CD25	0.1 mg	PC61

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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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