

Technical Data Sheet

Purified Mouse Anti-Mouse Pre-T Cell Receptor α Chain

Product Information

Material Number:	552407
Alternate Name:	pT α
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	2F5
Immunogen:	Recombinant extracellular (Ig-like) domain of pT α
Isotype:	Mouse (C57BL/6) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

At very early stages in the intrathymic differentiation of T lymphocytes which will bear $\alpha\beta$ T Cell Receptors (TCR), the TCR β chain forms a heterodimer with the pre-TCR α chain (pT α) to form the pre-TCR. This pre-TCR associates with the signal-transducing CD3 complex and controls the survival and proliferation of CD4- CD8- (DN) thymocytes, plays a role in allelic exclusion of the TCR β chain, directly or indirectly regulates TCR δ chain expression, and is eventually replaced by the TCR α chain as thymocytes mature. The 2F5 antibody reacts with the pT α on the surface of CD44- CD25-/lo DN thymocytes.

Preparation and Storage

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

Store undiluted at 4°C.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Reported

Recommended Assay Procedure:

This antibody has been tested by cell-surface immunofluorescent staining ($\leq 8\mu\text{g}/\text{million cells}$) with flow cytometric analysis to assure specificity and reactivity. Since the pT α is expressed at low density on the surface of immature thymocytes, we recommend the use of Mouse Fc Block™ (anti-mouse CD16/CD32 mAb 2.4G2, Cat. No. 553141/553142) and amplification of the staining intensity through the use of a biotinylated second-step antibody, such as anti-mouse IgG1 mAb A85-1 (Cat. No. 553441), followed by a "bright" third-step reagent, such as Streptavidin-PE (Cat. No. 554061).

Suggested Companion Products

Catalog Number	Name	Size	Clone
557273	Purified Mouse IgG1, κ Isotype Control	0.5 mg	MOPC-31C
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553441	Biotin Rat Anti-Mouse IgG1	0.5 mg	A85-1
554061	PE Streptavidin	0.5 mg	(none)
553133	FITC Rat Anti-Mouse CD44	0.5 mg	IM7
557192	APC Rat Anti-Mouse CD25	0.1 mg	PC61
553052	PerCP Rat Anti-Mouse CD4	0.1 mg	RM4-5
553036	PerCP Rat Anti-Mouse CD8a	0.1 mg	53-6.7

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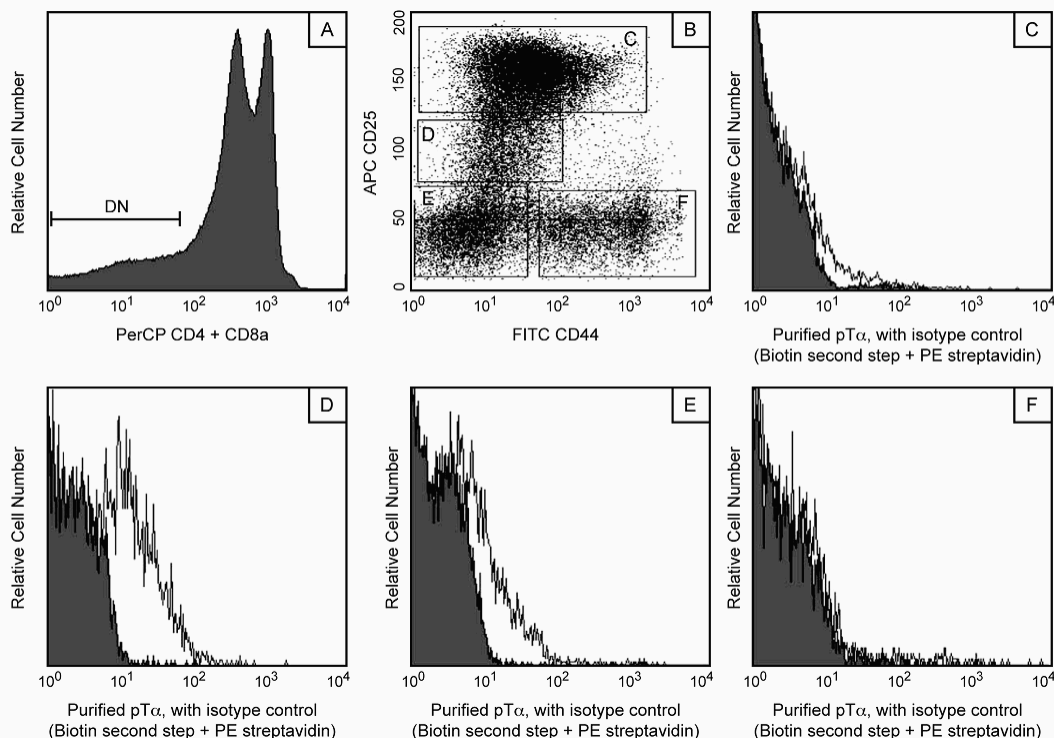
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Multicolor analysis of the expression of pTα on thymocyte subsets. C57BL/6 thymocytes were stained with either purified mAb 2F5 (top right panel, bottom panels, (C-F) open histograms) or purified mouse IgG1, κ isotype control MOPC-31C (Cat. No. 557273, filled histograms) in the presence of Mouse Fc Block™ (purified anti-mouse CD16/CD32 mAb, Cat. No. 553141/533142), followed by biotinylated anti-mouse IgG1 mAb A85-1 (Cat. No. 553441), then Streptavidin-PE (Cat. No. 554061). FITC-conjugated anti-mouse CD44 mAb IM7 (Cat. No. 553133) and APC-conjugated anti-mouse CD25 mAb PC61 (Cat. No. 557192) were added in a fourth step. PerCP-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553052) and anti-mouse CD8a mAb 53-6.7 (Cat. No. 553036) were included in the fifth staining step. For data analysis, CD4⁺ CD8a⁻ (DN) thymocytes were gated as indicated in the top left panel. The expression pattern of CD44 and CD25 in that DN population is displayed in the top middle panel. The remaining panels display histograms of pTα expression on the gated subpopulations of DN thymocytes: Panel C, CD44⁺/CD25^{hi}; Panel D, CD44⁺ CD25^{lo}; Panel E, CD44⁻ CD25⁺; and Panel F, CD44⁻ CD25⁻. Flow cytometry was performed on a BD FACSCalibur™ System (BD Biosciences, San Jose, CA).

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. 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

- Aifantis I, Azogui O, Feinberg J, Saint-Ruf C, Buer J, von Boehmer H. On the role of the pre-T cell receptor in alphabeta versus gammadelta T lineage commitment. *Immunity*. 1998; 9(5):649-688.(Immunogen: Immunoprecipitation)
- Aifantis I, Feinberg J, Fehling HJ, Di Santo JP, von Boehmer H. Early T cell receptor beta gene expression is regulated by the pre-T cell receptor-CD3 complex. *J Exp Med*. 1999; 190(1):141-144.(Biology)
- Aifantis I, Pivniouk VI, Gartner F, et al. Allelic exclusion of the T cell receptor beta locus requires the SH2 domain-containing leukocyte protein (SLP)-76 adaptor protein. *J Exp Med*. 1999; 190(8):1093-1102.(Biology)
- Mancini S, Candeias SM, Fehling HJ, von Boehmer H, Jouvin-Marche E, Marche PN. TCR alpha-chain repertoire in pTalpha-deficient mice is diverse and developmentally regulated: implications for pre-TCR functions and TCRA gene rearrangement. *J Immunol*. 1999; 163(11):6053-6059.(Biology)
- von Boehmer H, Aifantis I, Feinberg J, et al. Pleiotropic changes controlled by the pre-T-cell receptor. *Curr Opin Immunol*. 1999; 11(2):135-142.(Biology)
- von Boehmer H, Fehling HJ. Structure and function of the pre-T cell receptor. *Annu Rev Immunol*. 1997; 15:433-452.(Biology)
- Wiest DL, Berger MA, Carleton M. Control of early thymocyte development by the pre-T cell receptor complex: A receptor without a ligand. *Semin Immunol*. 1999; 11(4):251-262.(Biology)