Technical Data Sheet

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

Product Information

Material Number: 552407
Alternate Name: Ptcra; pTα; pTa; gp33; pT[a]; pTalpha; pT-alpha; pT-alpha-TCR
Size: 0.1 mg
Concentration: 0.5 mg/ml
Clone: 2F5
Immunogen: Recombinant extracellular (Ig-like) domain of pTα
Isotype: Mouse (C57BL/6) IgG1, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description
At very early stages in the intrathymic differentiation of T lymphocytes which will bear αβ T Cell Receptors (TCR), the TCR β chain forms a heterodimer with the pre-TCR α chain (pTα) 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 monoclonal antibody specifically recognizes the pTα 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

<table>
<thead>
<tr>
<th>Application</th>
<th>Routinely Tested</th>
<th>Reported</th>
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<tbody>
<tr>
<td>Flow cytometry</td>
<td></td>
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<tr>
<td>Immunoprecipitation</td>
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Recommended Assay Procedure:
This antibody has been tested by cell-surface immunofluorescent staining (≤8µg/million cells) with flow cytometric analysis to assure specificity and reactivity. Since the pTα 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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>557273</td>
<td>Purified Mouse IgG1, κ Isotype Control</td>
<td>0.5 mg</td>
<td>MOPC-31C</td>
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<tr>
<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.1 mg</td>
<td>2.4G2</td>
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<tr>
<td>553441</td>
<td>Biotin Rat Anti-Mouse IgG1</td>
<td>0.5 mg</td>
<td>A85-1</td>
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<tr>
<td>554061</td>
<td>PE Streptavidin</td>
<td>0.5 mg</td>
<td>(none)</td>
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<td>553133</td>
<td>FITC Rat Anti-Mouse CD44</td>
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<td>IM7</td>
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<tr>
<td>557192</td>
<td>APC Rat Anti-Mouse CD25</td>
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<td>PC61</td>
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<tr>
<td>553052</td>
<td>PerCP Rat Anti-Mouse CD4</td>
<td>0.1 mg</td>
<td>RM4-5</td>
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<tr>
<td>553036</td>
<td>PerCP Rat Anti-Mouse CD8α</td>
<td>0.1 mg</td>
<td>53-6.7</td>
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</table>
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. 553141/553142), 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-/lo CD25hi; Panel D, CD44- CD25lo; 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.
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
West 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)