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AM31862PU-N Monoclonal Antibody to T Cell Receptor (TCR) V alpha-2 - Purified

Quantity: 0.2 mg

Concentration: 0.2 mg/ml

Background: The TCR alpha chain complexes with the TCR beta chain to form the T cell receptor in 95% of T cells, whereas the remaining 5% of T cells express gamma and delta chains (γ/δ). TCR Vα2 is a distinct TCR subfamily found in mice having the a, b, and c haplotypes.

Host / Isotype: Rat / IgG2a

Recommended Isotype Controls: SM15P, SM15PX

Clone: B20.1

Format:
State: Liquid purified IgG fraction.
Purification: Protein G Affinity Chromatography.
Buffer System: PBS containing 0.09% Sodium Azide as preservative.

Applications:
Flow Cytometry (See Protocols).
This clone has also been reported to work in **Immunoprecipitation.** (1,2)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with Mouse T-Cell Receptor (TCR) Vα2 chains (1), and recognizes the majority of the TCR Vα2 subfamily in mice carrying the a, b and c haplotypes 1,2. It also reacts with the products of T cell receptor, Vδ8 due to the high degree of homology (1).
Species: Mouse.
Other species not tested.

Storage: Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:
1. Pircher, H., N. Rebai, M. Groettrup, C. Gregoire, D. E. Speiser, M. P. Happ, E. Palmer, R. M. Zinkernagel, H. Hengartner, B. Malissen. 1992. Eur. J. Immunol. 22:399-404.
2. Gregoire, C., N. Rebai, F. Schweisguth, A. Necker, G. Mazza, N. Auphan, A. Millward, Anne-Marie Schmitt-Verhulst, B. Malissen. 1991. Proc. Natl. Acad. Sci. USA. 88:8077-8081.

Protocols:
<u>Flow Cytometry Analysis:</u>
NOTE: Preblocking of Fc receptors for 10 minutes using 0.5 µg of purified anti-Mouse CD16/32 is recommended.

Method:
1. Prepare cell suspension in Media A. For cell reparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.

3. Resuspend the cells to a concentration 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing one test).
4. To each tube add 0.25 µg of this antibody AM31862PU-N per 1x10e6 cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-Rat IgG (H+L) at a 1/500 dilution.
9. Incubate tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in Media B.
11. Resuspend the cell pellet in 50 µl ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in phosphate buffered saline. This stains dead cells by intercalating DNA.

Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2 M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls).