

AM31862BT-N **Monoclonal Antibody to T Cell Receptor (TCR) V alpha-2 - Biotin**

Quantity:	0.1 mg
Concentration:	0.1 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 Va2 is a distinct TCR subfamily found in mice having the a, b, and c haplotypes.
Host / Isotype:	Rat / IgG2a
Clone:	B20.1
Format:	State: Liquid purified IgG fraction. Purification: Protein G Affinity Chromatography. Buffer System: PBS containing 0.09% Sodium Azide as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. Label: Biotin
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) Va2 chains (1), and recognizes the majority of the TCR Va2 subfamily in mice carrying the a, b and c haplotypes 1,2. It also reacts with the products of T Cell Receptor, V δ 8 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:	Flow Cytometry Analysis: NOTE: Preblocking of Fc receptors for 10 minutes using 0.5 μ g of purified anti-Mouse CD16/32 is recommended. Method: 1. Prepare cell suspension in Media A. For cell replications, deplete the red blood cell population with Lympholyte®-M cell separation medium. 2. Wash 2 times.

3. Resuspend the cells to a concentration 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing one test).
4. To each tube add $\sim 0.25 \mu\text{g}^*$ of this antibody AM31862BT-N per 1×10^6 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 μ l 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 μ l ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l 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 μ l of 2 M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 μ l of 2 M sodium azide in 100 mls).