

MONOCLONAL ANTIBODY

V β 12

Cat. No.	Form	Quantity	Presentation
1583	Purified	0.1 mg	Freeze-dried
1587	FITC	50 tests	Liquid 1 mL
2019	Biotin	0.1 mg	Freeze-dried
2291	PE	50 tests	Liquid 1 mL

Clone	VER2.32.1
Isotype	IgG2a (mouse)
Immunogen	Mouse T cell hybridoma transfected with human V β 12 gene segment.
Hybridoma	X63 Ag8.653 x Balb/c spleen cells
Specificity	<p>Human variable β12 chain of the T cell receptor also called TCRBV12S2 according to the nomenclature from Wei et al (1).</p> <p>The Vβ12 subfamily has 3 members (1).</p> <p>VER2.32.1 recognizes the Vβ12.2 sequence HBP54 (2), which is identical to PH27 (3). Reactivity with other members of the Vβ12 subfamily has not been found, but cannot be formally excluded.</p> <p>A cell line derived from PBL by sorting with the antibody is 100% recognized by the formerly described antibody S511 anti-Vβ12 (4) and vice versa. No cross reactivity could be detected on the VER2.32.1 sorted cells by any of 18 antibodies specific for different Vβ chains (covering 60-70% of the $\alpha\beta$ repertoire) (5).</p> <p>On the average, this antibody stains 1.5% (sd=0.3) of peripheral CD3 positive lymphocytes from 20 healthy adult donors (data on file at Immunotech).</p> <p>The specificity of this antibody has been confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (5).</p>
Applications	<p>T cell repertoire studies in normal and in pathological situations, including autoimmune diseases, chronic inflammatory diseases, cancer, bone marrow transplantation, graft rejection and AIDS.</p> <p>Vβ12 is the target of the staphylococcus enterotoxin B (SEB) superantigens and streptococcal pyrogenic exotoxin A (SPEA) (6).</p>
Buffer	<p>Freeze-dried forms: 1 mg/mL bovine serum albumin in phosphate-buffered saline.</p> <p>Liquid forms: 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.</p>

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MA003

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES

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Reconstitution and Storage

The freeze-dried form may be stored at 2-8°C until the expiration date. Reconstitute with 0.5 mL of distilled water. No preservative has been added. The reconstituted form may be stored at -20°C until the expiration date. Aliquotting is suggested to avoid multiple freeze-thaw cycles. The addition of sodium azide at 0.1% (w/v) is recommended for storage of the reconstituted form for up to one month at 2-8°C.

The conjugated forms should not be frozen and should be stored in the dark at 2 - 8°C.

Recommended Procedures

Fluorescent microscopy or flow cytometry:

Liquid form: 20 µL / 5×10^5 cells / test or 100 µL whole blood

Freeze-dried form: 2 µg / 5×10^5 cells / test or 100 µL whole blood

Since this antibody recognizes a small cell population, it is often preferable to use double staining experiments with another T cell marker (CD2, CD3, CD4, CD8, etc.). Double staining is also possible with the purified unlabelled form using the following protocol.

A. Double labelling protocol using the freeze-dried unconjugated form (Cat. No. 1583) with CD3 PE (Cat. No. 1282)

1. In 100 µL of whole blood, add 10 µL of the reconstituted purified antibody. Incubate 15 minutes at room temperature (18-25°C).
2. Add 3 mL of PBS/BSA/NaN₃. Centrifuge 5 minutes 1200 rpm, discard supernatant.
3. Add 100 µL of secondary antibody F(ab')₂ goat anti-mouse Ig conjugated to FITC (Cat. No. 0819) at usual dilution in PBS/BSA NaN₃. Incubate 15 minutes at room temperature.
4. Repeat step 2 (washing).
5. Resuspend cells in 100 µL of PBS/BSA/NaN₃ containing 1 mg/mL of total mouse Ig (to saturate eventual free sites of the goat anti-mouse FITC). Incubate 5 minutes at room temperature.
6. Without washing, add 20 µL of the CD3 PE (Cat.No. 1282). Incubate 15 minutes at room temperature.
7. Repeat step 2 (washing).
8. Proceed as usual for lysis of red blood cells and fixing of white cells.

B. Double labelling protocol using biotinylated form (Cat. No. 2019) with CD3 PE (Cat. No. 1282)

1. To 100 µL of whole blood add 10 µL of the reconstituted biotinylated form, and 20 µL of CD3 PE. Incubate 15 minutes at room temperature.
2. Add 3 mL PBS/BSA/NaN₃. Centrifuge 5 minutes at 1200 rpm, discard supernatant.
3. Add 100 µL of FITC conjugated streptavidin at the usual recommended dilution.
4. Repeat step 2.
5. Then proceed as usual for lysis of red blood cells and fixing of white cells.

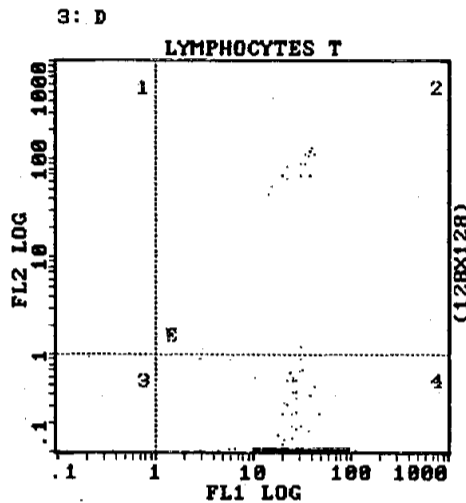
C. Double labelling protocol using the PE conjugated form (Cat. No. 2291) with CD3 FITC (Cat. No. 1281)

1. To 100 μ L of whole blood add 20 μ L of PE conjugate and 20 μ L of CD3 FITC. Incubate 15 minutes at room temperature.
2. Add 3 mL of PBS/BSA/NaN₃. Centrifuge 5 minutes at 1200 rpm, discard supernatant.
3. Proceed as usual for lysis of red blood cells and fixing of white cells.

NOTE: PBS/BSA/NaN₃ = PBS/BSA 0.2% / NaN₃ 0.02%.

Example data

Flow cytometric analysis of a typical double staining experiment CD3 FITC / V β 12 PE (gating on CD3⁺ lymphocytes).



Quadrant 2: CD3⁺ - V β 12⁺
 Quadrant 4: CD3⁺ - V β 12⁻

References

- 1) Wei, S., Charmley, P., Robinson, M.A., Concannon, P., "The extent of the human germline T-cell receptor V beta gene segment repertoire", 1994, *Immunogenetics*, **40**, 27-36.
- 2) Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debré, P., Minden, M., Mak, T., "Sequences and diversity of human T-cell receptor β chain variables region genes", 1986, *J. Exp. Med.*, **164**, 739-750.
- 3) Tillinghast, J.P., Behlke, M.A., Loh, D.Y., "Structure and diversity of the human T-cell receptor beta chain variable region genes", 1986, *Science*, **22**, 879-883.
- 4) Bigler, R.D., Fisher, D.E., Wang, C.Y., Rinnooy Kan, E.A., Kunkel, H.G., "Idiotypic-like molecules on cells of a human T-cell leukemia", 1983, *J. Exp. Med.*, **158**, 1000-1005.
- 5) Posnett, D.N., Romagné, F., Necker, A., Kotzin, B.L., Sekaly, R.P., "First Human TcR Monoclonal Antibody Workshop", 1996, *The Immunologist*, **4**, (1), 5-8.
- 6) Fleischer, B., Necker, A., Leget, C., Malissen, B., Romagné, F., "Reactivity of mouse T-cell hybridomas expressing human V β gene segments with staphylococcal superantigens", 1996, *Infection and Immunity*, **64**, (3), 987-994.

