

MONOCLONAL ANTIBODY V β 22

Cat. No.	Form	Quantity	Presentation
1365	Purified	0.1 mg	Freeze-dried
1484	FITC	50 tests	Liquid 1ml
2026	Biotin	0.1 mg	Freeze-dried
2051	PE	50 tests	Liquid 1 ml

Clone IMMU 546

Isotype IgG1 (mouse).

Immunogen Murine T-cell hybridoma transfected with human V β 22 gene segment.

Hybridoma Myeloma X63 Ag 8 653 X Balb/c spleen cells.

Specificity Human variable β 22 of human T-cell receptor also called TCRBV22S1 according to the nomenclature from Wei et al (1).

This antibody recognizes at least the IGRb03 sequence (2)

This antibody has been further characterized by cell sorting on PBL using this monoclonal antibody followed by analysis of sorted cells by molecular biology.

Analysis of α chain mRNA by PCR with a panel of α specific oligonucleotides shows transcripts for most V α sequences.

Analysis of β chain mRNA by anchored PCR and sequencing, only shows transcripts for IGRb03 sequence.

There is another V β 22 sequence differing only by one amino acid, MARVB23 (3). Though very similar this sequence has not been found by the technique described above until now. If the antibody either does not recognize the other V β 22 sequence, or if this sequence is of very low occurrence remains to be determined. It could also be an allelic difference. However, as IMMU 546 recognizes a stable percentage in PBL from many donors, it is not likely this monoclonal antibody is specific for an allelic form.

This antibody recognizes 2.4 to 5.1% of peripheral CD3 positive lymphocytes from 6 healthy adult donors (data on file at Immunotech).

The specificity of this antibody has been confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (6).

Applications

Studies have shown that anti-V β 22 may be useful in T-cell repertoire studies in normal and pathological situations including autoimmune diseases particularly rheumatoid arthritis (4), graft rejection or AIDS (5).

Superantigenic stimulation of T cells.

May 23, 1996

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Buffer Freeze-dried forms: 1 mg/ml bovine serum albumin in phosphate buffered saline
Liquid forms: 2 mg/ml bovine serum albumin in phosphate buffered saline containing 0.1% sodium azide.

Reconstitution and Storage The freeze-dried form may be stored at 2-8°C until the expiration date. Reconstitute with 0.5 ml of d.stilled water. No preservative has been added. The reconstituted form may be stored at -20°C until the expiration date. Aliquoting 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

Flow cytometry

Freeze-dried form: 2 µg/5x10⁵ cells/test
Liquid form: 20 µl/5x10⁵ cells/test or 100 µl whole blood.

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

A. Double labelling protocol using freeze-dried unconjugated form (Cat. No. 1365) with CD3 PE (Cat. No 1282)

1. To 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 at 1200 rpm, discard supernatant.
3. Add 100 µl of secondary antibody F(ab')₂ goat anti-mouse Ig conjugated to FITC at usual dilution in PBS/BSA/NaN₃ (Cat No. 0819) 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. 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. 2026) with a CD3 FITC (Cat. No. 1281).

1. To 100 µl of whole blood add 20 µl of the reconstituted biotinylated form and 20 µl of the CD3 FITC. Incubate 15 minutes at room temperature.

DATA SHEET

Cat No 1365

- 2 Add 3 ml of PBS/BSA/NaN₃. Centrifuge 5 minutes at 1200 rpm, discard supernatant.
 - 3 Add 100 µl of PE conjugated streptavidine at the usual dilution
 4. Repeat step 2.
 - 5 Then 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%

C. Double labelling protocol using PE conjugated form, (Cat. No. 2051) with a CD3 FITC (Cat No. 1281)

1. To 100 µl of whole blood add 20 µl of the PE conjugate and 20 µl of CD3 FITC Incubate 15 minutes at room temperature
 2. Add 3 ml 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%

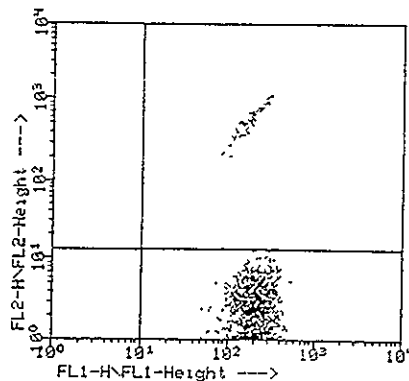
Example Data Flow cytometric analysis of a typical double staining experiment CD3 FITC / Vβ22 PE (gating on CD3⁺ lymphocytes).

----- Quad Stats -----
File: U3:UB213P10 Sample: 3FITC/UB22PE D1 010
Date: 4/ 2/96 Gate G2= R2
Selected Preference: Arithmetic/Linear
Parameters: FL1-H(LOG),FL2-H(LOG) Quad Location: 10.00,13.34
Total= 8217 Gated= 1984
Quad Events % Gated % Total Xmean Ymean

Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	96	4.84	1.17	179.63	566.63
3 LL	0	0.00	0.00	--	--
4 LR	1888	95.16	22.98	215.35	2.45

Quadrant 2: CD3⁺ Vβ22⁺

Quadrant 4: CD3⁺ Vβ22⁻



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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) Ferradini, L., Roman-Roman, S., Azocar, J., Michalaki, H., Triebel, F., Hercend, T., "Studies on the human TCR alpha beta variable region genesII. Identification of four additional V beta subfamilies", 1991, Eur. J. Immunol., **21**, 935-942.
- 3) Robinson, M.A., "The human TCR beta chain gene complex contains at least 57 variable gene segments", 1991, J Immunol., **146**, 4392-4397.
- 4) Imberti, L., Sottini, A., Bettinardi, A., Puoti, M., Primi, D., "Selective depletion in HIV infection of T cells that bear specific T cell receptor V β sequences', 1991, Science, **254**, 860-862.
- 5) Marrack, P., Kappler, J., "The staphylococcal enterotoxins and their relatives", 1990, Science, **248**, 705-710
- 6) 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.