MONOCLONAL ANTIBODY Vβ17

Cat. No.	Form	Quantity	Presentation
1189	Purified	0.1 mg	Freeze-dried
1194	Biotin	0.1 mg	Freeze-dried
1234	FITC	50 tests	Liquid 1 ml
2048	PE	50 tests	Liquid 1 ml

Clone E17.5F3

Isotype IgG1 (mouse)

Immunogen Murine T-cell hybridoma transfected with Vβ17 gene segment.

Hybridoma Myeloma X63 Ag 8.653 X Balb/c spleen cells.

Specificity Human variable β17 chain of the T-cell receptor also called TCRBV17S1 according to the nomenclature of Wei et al.(1). Vβ17 is a single membered family (HBVT02 (2)) This antibody has been further characterized by cell sorting on PBL using this antibody followed by analysis of sorted cells by molecular biology (3,4).

Analysis of α chain mRNA by PCR with a panel of a specific oligonucleotides shows transcripts for most Vα sequences.
Analysis of β chain mRNA by anchored PCR and sequencing, only shows transcripts for β17 gene segment (HBVT02 sequence).

The antibody stains 3.3 to 7% of peripheral CD3 positive lymphocytes from 6 healthy adult donors (data on file at Immnotech).

This antibody is described in Ref. 4
The specificity of this antibody has been confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (5).

Applications Studies have shown that Vβ17 may be useful in T-cell repertoire studies in normal and pathological situations including autoimmune disease, particularly rheumatoid arthritis (6) and AIDS (7).

Superantigenic stimulation of T cells; Vβ17 seems to be the target of MAM (6) (Mycoplasma Arthritis derived superantigen)

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

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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

Flow cytometry:

Liquid form: 20 µl//5x10^5 cells/test

Freeze-dried form: 2 µg/5x10^5 cells/test

Since this antibody recognizes a small 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 the purified unlabelled antibody form using the following protocols.

A. Double labelling protocol using freeze dried unconjugated form (Cat. No. 1189) with a 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.

2. Add 3 ml of PBS/BSA/NaN_3. 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 recommended dilution in PBS/BSA/NaN_3 Incubate 15 minutes at room temperature

4. Repeat step 2 (washing).

5. Resuspend cells in 100 µl of PBS/BSA/NaN_3 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

Cat. No 1189

B Double labelling protocol using biotinylated form, (Cat. No 1194) with a 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/NaN_3. Centrifuge 5 minutes at 1200 rpm, discard supernatant.

3 Add 100 µl of FITC conjugated streptavidine at the usal 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 PE conjugated form, (Cat. No 2048) with a CD3 FITC (Cat No 1281)

1. In 100 µl of whole blood add 20 µl of Vβ17 PE conjugate and 20 µl of CD3 FITC Incubate 15 minutes at room temperature.

2. Add 3 ml of PBS/BSA/NaN_3. 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/NaN_3 = PBS/BSA 0.2%/NaN_3 0.02%.

Example Data Flow cytometric analysis of a typical double staining experiment CD3 FITC / Vβ17 PE (gating on $CD3^+$ lymphocytes).

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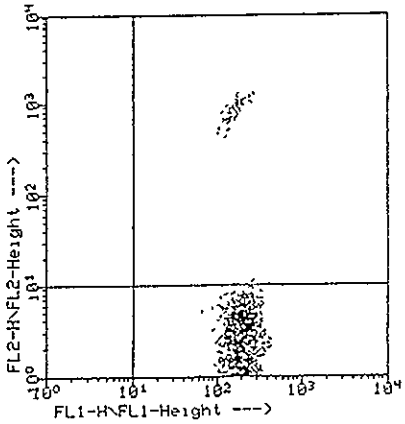
-----  Quad Stats  -----
File: 11:VB171839  Sample: 3FITC/VB17PE          D2 039
Date:  3/ 1/96  Gate G2= R2
Selected Preference: Arithmetic/Linear
Parameters: FL1-H(LOG),FL2-H(LOG)  Quad Location: 10.00,10.00
Total=     7851  Gated=     1923
```

Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	124	6.45	1.58	167.32	798.36
3 LL	0	0.00	0.00	--	--
4 LR	1799	93.55	22.91	201.51	2.73

Quadrant 2 : $CD3^+$ - $V\beta17^+$

Quadrant 4 : $CD3^+$ - $V\beta17^-$



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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.

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3) Diu, A., Romagné, F., Genevée, C., Rocher, C., Bruneau, J.M., David, A , Praz, F., Hercend, T., "Fine specificity of monoclonal antibodies directed at human T cell receptor variable regions: comparison with oligonucleotide driven amplification for evaluation of V beta expression", 1993, Eur. J. Immunol., **23**, 1422-1429.

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7) Imberti, L., Sottini, A., Bettinardi, A., Puoti, M , Primi, D., "Selective depletion in HIV infection of T cells that bear specific T cell receptor Vβ sequences', 1991, Science, **254**, 860-862.