

MONOCLONAL ANTIBODY V β 18

Cat. No.	Form	Quantity	Presentation
1261	Purified	0.1 mg	Freeze-dried
2049	PE	50 tests	Liquid 1 ml

Clone	BA62
Isotype	IgG1 (mouse)
Immunogen	Human T-cell clone.
Hybridoma	Myeloma NS1 x Balb/c spleen cells.
Specificity	<p>Human variable β18 chain of the T-cell receptor, also called TCRBV18S1 according to the nomenclature from Wei et al (4). Vβ18 is a single membered family (PH29 sequence (1)) identical to HBVT56 (2). This antibody has been characterized by cell sorting on PBL using this monoclonal antibody, followed by molecular biology analysis of the sorted cells (3).</p> <p>Analysis of α chain mRNA using a panel of α specific oligonucleotides shows transcripts for most Vα sequences. Analysis of β chain mRNA by anchored PCR and sequencing only shows transcripts for PH29 sequence.</p> <p>This antibody stained 0.7 to 2.2% of peripheral CD3 positive lymphocytes from 7 healthy adult donors (data on file at Immunotech S.A.). Care must be taken in view of very low occurrence of Vβ18⁺ cells. Double staining with anti-CD2 or CD3 is preferable. Study of the sorted cells shows a heterogeneous pattern of staining with this antibody and one T cell clone typed by PCR to be Vβ18 positive is not stained. This antibody may therefore be sensitive to the "clonotypic environment" and may not pick up all Vβ18 positive T cells.</p> <p>This specificity formerly called Vβ17 by Immunotech according to the nomenclature from Wilson (5) was confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (6).</p>
Applications	T-cell repertoire studies in normal and pathological situations including autoimmune disease, graft rejection or AIDS.
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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MA001

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 ProceduresFlow cytometry:

Liquid form: 20 µl/5x10⁵ cells/test

Freeze-dried form: 2 µg/5x10⁵ 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 purified unlabelled antibody form using the following protocol.

A - Double labelling protocol using freeze-dried unconjugated form (Cat.No.1261) 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/NaN₃. Centrifuge 5 minutes 1200 rpm, discard supernatant.
3. Add 100 µl of secondary antibody Fab'2 goat anti-mouse Ig conjugated to FITC 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. 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.

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

B - Double labelling protocol using PE conjugated form, (Cat. No. 2049) with CD3 FITC (Cat No. 1281).

1. In 100 µl of whole blood add 20 µl of Vβ18 PE conjugate and 20 µl of CD3 FITC conjugate. 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.

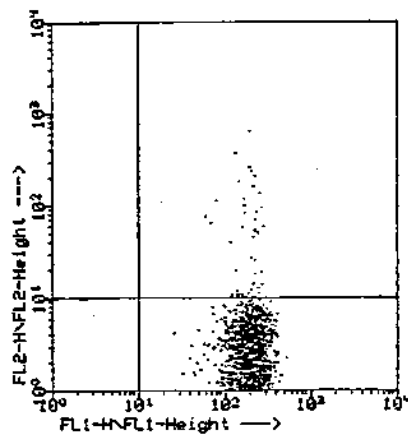
Note: PBS/BSA/NaN₃ = PBS/BSA 0.2%/NaN₃ 0.02%.

Example Data. Flow cytometric analysis of a typical double staining experiment CD3 FITC / Vβ18 PE (gating on CD3⁺ lymphocytes).

----- Quad Stats -----

File: 13:UB171818 Sample: 3FITC/UB18PE D2 018
 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= 9166 Gated= 2020

Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	45	2.23	0.49	137.22	88.37
3 LL	0	0.00	0.00	--	--
4 LR	1975	97.77	21.55	199.99	2.65



Quadrant 2: CD3⁺ Vβ18⁺
 Quadrant 4: CD3⁺ Vβ18⁻

References

- 1) 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*, **233**, 879-883.
- 2) Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R.P., Mak, T.W., "Sequences and repertoire of the human T cell receptor alpha and beta chain variable region genes in thymocytes", 1987, *Eur. J. Immunol.*, **17**, 375-383.
- 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.
- 4) 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.
- 5) Wilson, R.K., Lai, E., Concannon, P., Barth, R.K., Hood, L.E., "Structure, organisation and polymorphism of murine and human TCR alpha and beta chain gene families", 1988, *Immunol. Rev.*, **101**, 149-172.
- 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.

