

MONOCLONAL ANTIBODY V β 5.3

Cat. No.	Form	Quantity	Presentation
1370	Purified	0.1 mg	Freeze-dried
2002	PE	50 tests	Liquid 1 ml

Clone	3D11
Isotype	IgG1 (mouse)
Immunogen	HPB ALL
Hybridoma	X63.Ag8.653 x Balb/c spleen cells
Specificity	Human variable β 5.3 chain of the T-cell receptor, also called TCRBV5S3 according to the nomenclature from Wei et al. (1)

This antibody recognizes the IGRb08 sequence (2), identical to 12A1 sequence (3). It does not recognize V β 5.2 (PL2.5 sequence (4)) and V β 5.1 (HBP51 sequence (5)). Recognition of other members of the V β 5 family has not been detected but cannot be formally excluded.

Cell sorting of PBL gives a homogeneous population that is not recognized by 18 monoclonal antibodies specific for different V β chains (which make up around 70% of the α/β repertoire).

Various D β , J β and V β sequences are found associated with V β 5.3 on sorted cells when analysed by molecular biology.

3D11 stains from 1 to 1.8% of CD3 positive lymphocytes from 6 healthy adult donors (data on file at Immunotech).

3D11 is described in (6).

Applications T-cell repertoire studies in normal and pathological situations including autoimmune disease, particularly Rheumatoid Arthritis (7), graft rejection or AIDS (8).

Superantigenic stimulation of T cells.

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. 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 ProceduresFlow cytometry:

Liquid form: 20 µl/5x10⁵ cells/test or 100 µl whole blood.

Freeze-dried form: 2 µg/5x10⁵ cells/test.

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.1370) 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 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.

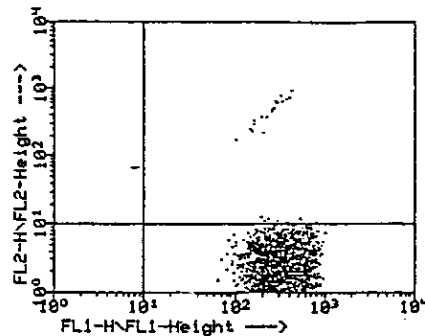
B. Double labelling protocol using the PE conjugated form (Cat. No.2002) with CD3 FITC (Cat. No.1281).

1. To 100 µl of whole blood add 20 µl of PE conjugate and 20 µl 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 0.2%

Example Data

Flow cytometric analysis of a typical double staining experiment CD3 FITC/Vβ5.3 PE (gating on CD3⁺ lymphocytes).



----- Quad Stats -----
 File: U3:UB53PE21 Sampler 3/UB5.3PE D1 021
 Date: 12/21/95 Gate G2= R2
 Selected Preference: Arithmetic/Linear
 Parameters: FL1-H(LOG),FL2-H(LOG) Quad Location: 10.00,10.37
 Total= 16973 Gated= 2629

Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	25	0.95	0.15	261.55	347.31
3 LL	0	0.00	0.00	--	--
4 LR	2504	99.05	15.34	340.27	2.44

Quadrant 2: CD3⁺ - Vβ5.3⁺
 Quadrant 4: CD3⁺ - Vβ5.3⁻

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 genes. Identification of four additional V beta subfamilies", 1991, Eur. J. Immunol., **21**, 935-942.
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- 4) Concannon, P., Pickering, L., Kung, P., Hood, L., "Diversity and structure of human T-cell receptor beta-chain variable region genes", 1986, Proc. Natl. Acad. Sci. USA, **83**, 6598-6602.
- 5) Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debre, P., Minden, M.D., Mak, T.W., "Sequences and diversity of human T cell receptor beta chain variable region genes", 1986, J. Exp. Med., **164**, 739-750.
- 6) Carrel, S., Isler, P., Schreyer, M., Vacca, A., Salvi, S., Giuffre, L., Mach, J., "Expression on human thymocytes of the idiotypic structures (Ti) from two leukemia T cell lines Jurkat and HPB-ALL", 1986, Eur. J. Immunol., **16**, 649-652.
- 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.
- 8) Marrack, P., Kappler, J., "The staphylococcal enterotoxins and their relatives", 1990, Science, **248**, 705-710.