

MONOCLONAL ANTIBODY

Vβ1

Cat. No.	Form	Quantity	Presentation
2219	Purified	0.1 mg	Freeze-dried
2355	PE	50 tests	Liquid 1 mL
2406	FITC	50 tests	Liquid 1 mL

Clone BL37.2

Isotype IgG1 (rat)

Immunogen Rat cell line RBL-2H3 transfected with the human TCR-Vβ1 gene.

Hybridoma Myeloma X63-Ag8.653 x Lou rat spleen cells

Specificity Human variable β1 chain of the T-cell receptor (Vβ1.1) also called TCRBV1S1 according to the nomenclature from Wei et al. (1).
 BL37.2 recognizes both Vβ1.1 and Vβ1.2 allele products (HBVT73 cDNA, in ref. 2, and 46W/Q cDNA, in ref. 3, respectively). None of the other Vβ specific antibodies from the First TCR Workshop (San Francisco, CA, 1995) gave significant staining on the sorted cell lines (4).
 This antibody stains from 2.4 to 6.6% of peripheral CD3-positive lymphocytes from 6 healthy donors (data on file at Immunotech).

Applications T-cell repertoire studies

Buffer Freeze-dried form: 1 mg/mL bovine serum albumin in phosphate-buffered saline.
 Liquid form: 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 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 until the expiration date stated on the vial label.

Recommended Procedures Flow cytometry
 Freeze-dried form: 2 µg / 5 x 10⁵ cells / test.
 Liquid form: 20 µL / 5 x 10⁵ cells / test or µL whole blood

As 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

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staining is also possible with purified unlabelled antibody form using the following protocol.

A. Double labelling protocol using Vβ1 freeze-dried unconjugated form (Cat. No. 2219) 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 (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. 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 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.

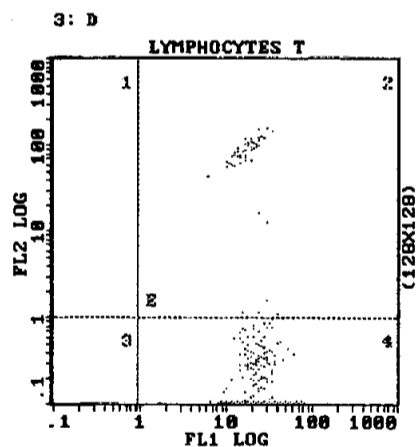
B. Double labelling protocol using conjugated forms (Cat. No. 2355 or 2406) with CD3 conjugated (Cat. No. 1281 or 1282)

1. To 100 μL of whole blood add 20 μL of Vβ1 conjugate and 20 μL of CD3 conjugate. 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 / Vβ1 PE (gating on CD3⁺ lymphocytes).



Quadrant 2: CD3⁺ - Vβ1⁺
 Quadrant 4: CD3⁺ - Vβ1⁻

References

- 1) Wei, S., Chamley, P., Robinson, M.A., Concannon, P., "The extent of the human germline T-cell receptor V β gene segment repertoire", 1994, Immunogenetics, **40**, 27-36.
- 2) Robinson, M.A., "Allelic sequence variations in the hypervariable region of a T-cell receptor β chain: correlation with restriction fragment length polymorphism in human families and populations"; 1989, Proc. Natl. Acad. Sci. USA, **86**, 9422-9426.
- 3) Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R.P., Mak, T.W., "Sequences and repertoire of the human T-cell receptor α and β chain variable region genes in thymocytes, 1987, Eur. J. Immunol., **17**, 375-383.
- 4) 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.

