

MONOCLONAL ANTIBODY

Vβ7

Cat. No.	Form	Quantity	Presentation
2037	Purified	0.1 mg	Freeze-dried
2287	PE	50 tests	Liquid 1 mL
2288	Biotin	0.1 mg	Freeze-dried
2408	FITC	50 tests	Liquid 1 mL

- Clone** ZOE
- Isotype** IgG2a (mouse)
- Immunogen** Murine T-cell hybridoma transfected with Vβ7 gene segment.
- Hybridoma** Myeloma X63 Ag 8.653 x Balb/c spleen cells
- Specificity** Human variable β7 chain of the T-cell receptor also called TCRBV7S1 according to the nomenclature of Wei et al (1).
The ZOE antibody recognizes Vβ7.1 (PL4.9 sequence, 2). The reactivity on the other members Vβ7.2 and Vβ7.3 (2, 3) cannot be formally excluded.
On the average, this antibody stains 1.9% (sd=0.5) of peripheral CD3-positive lymphocytes from 20 healthy donors (data on file at Immunotech).
- Applications** T-cell repertoire studies in normal and in pathological situations, including autoimmune diseases, chronic inflammatory diseases, cancer, bone marrow transplantation, graft rejection and AIDS.
- 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 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.
- Recommended Procedures** Flow cytometry:
Freeze-dried form: 2 µg / 5 x 10⁵ cells / test or 100 µL whole blood
Liquid form: 20 µL / 5 x 10⁵ cells / test or 100 µL whole blood
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.).

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MA003

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES



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Double staining is also possible with the purified unlabelled form using the following protocols.

A. Double labelling protocol using the freeze-dried unconjugated form (Cat. No. 2037) with CD3 PE (Cat. No. 1282)

1. To 100 μ L of whole blood, add 10 μ L 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 μ L 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 μ L 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 μ L 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 biotinylated form (Cat. No. 2288) with CD3 FITC (Cat. No. 1281)

1. To 100 μ L of whole blood add 10 μ L of the reconstituted biotinylated form, and 20 μ L 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. Add 100 μ L of PE conjugated streptavidin at the usual recommended dilution.
4. Repeat step 2.
5. Then proceed as usual for lysis of red blood cells and fixing of white cells.

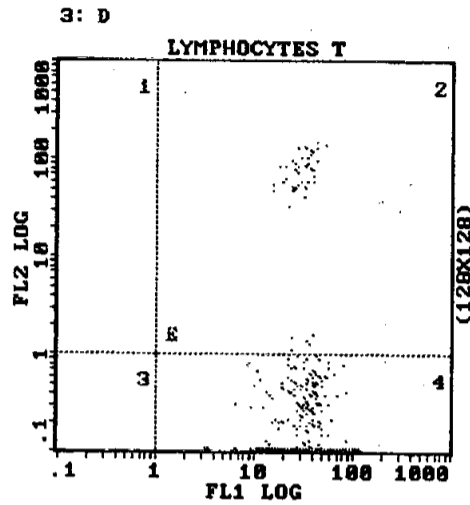
C. Double labelling protocol using the liquid conjugated forms (Cat. No. 2287 or 2408) with CD3 (Cat. No. 1281 or 1282)

1. To 100 μ L of whole blood add 20 μ L of V β 7 conjugate and 20 μ L of CD3 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 using CD3 FITC / Vβ7 PE (gating on CD3⁺ lymphocytes).



Quadrant 2: CD3⁺ Vβ7⁺
 Quadrant 4: CD3⁺ Vβ7⁻

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) Concannon, P., Pickering, L.A., Kung, P., Hood, L., "Diversity and structure of human T-cell receptor β-chain variable region genes", 1986, *Proc. Natl. Acad. Sci. USA*, **83**, 6598-6602.
- 3) Zhao, T.M., Whitaker, S.E., Robinson, M.A., "A genetically determined insertion/deletion related polymorphism in human T cell receptor beta chain (TCRB) includes functional variable gene segments", 1994, *J. Exp. Med.*, **180**, 1405-1414.

