

MONOCLONAL ANTIBODY

V β 3

Cat. No.	Form	Quantity	Presentation
2008	Purified	0.1 mg	Freeze-dried
2109	Biotin	0.1 mg	Freeze-dried
2284	PE	50 tests	Liquid 1 mL
2372	FITC	50 tests	Liquid 1 mL

Clone	CH92
Isotype	IgM (Mouse)
Immunogen	Human T-cell clone A2.
Hybridoma	Myeloma NS1 x Balb/c spleen cells.
Specificity	<p>Human variable β3 chain of the T-cell receptor also called TCRBV3S1 according to the nomenclature from Wei et al (1).</p> <p>The antibody recognizes the only member sequence (PL4.4 (2) or HBVT22 (3)) of this family.</p> <p>It should be noted that the level of human Vβ3 expression correlates with allelic polymorphism in the spacer region of the recombination signal sequence (4).</p> <p>This antibody has been characterized by cell sorting on PBL, followed by molecular analysis of the sorted cells (5).</p> <p>Analysis of α chain mRNA using a panel of α specific oligonucleotides shows transcripts for most Vα sequences.</p> <p>Analysis of β chain mRNA by anchored PCR and sequencing only shows transcripts for PL4.4 and HBVT22 sequences.</p> <p>From the TCR workshop held in San Francisco (July 1995), in contrast to the LE89 clone, this antibody recognizes most of BV3 positive cells (6, 7).</p> <p>On the average, CH92 stains 2.9% (sd=1.6) of peripheral CD3 positive lymphocytes from 20 healthy adult donors (data on file at Immunotech).</p>
Applications	<p>T-cell repertoire studies in normal and pathological situations including autoimmune diseases, chronic inflammatory diseases, cancer, bone marrow transplantation, graft rejection or AIDS.</p> <p>Superantigenic stimulation of T cells. Vβ3 is the target of SEB (8) and MAM (Mycoplasma Arthritis derived superantigen) (9). Recently, Yersinia Pseudotuberculosis mitogen, YPM, has also been shown to target Vβ3 (10, 11).</p>

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MA003

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES

**IMMUNOTECH**

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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. 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 until the expiration date stated on the vial label.

Recommended Procedures Flow cytometry:
Liquid form: 20 µL / 5×10^5 cells / test or 100 µL whole blood
Freeze-dried form: 2 µg / 5×10^5 cells / test or 100 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 staining is also possible with purified unlabelled antibody form using the following protocols.

A. Double labelling protocol using Vβ3 Purified (Cat. No. 2008) with 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 F(ab')₂ goat anti-mouse Ig conjugated to FITC (Cat. No. 0819) 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 and 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. 2109) with CD3 FITC (Cat. No. 1281)

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

Cat. No. 2008

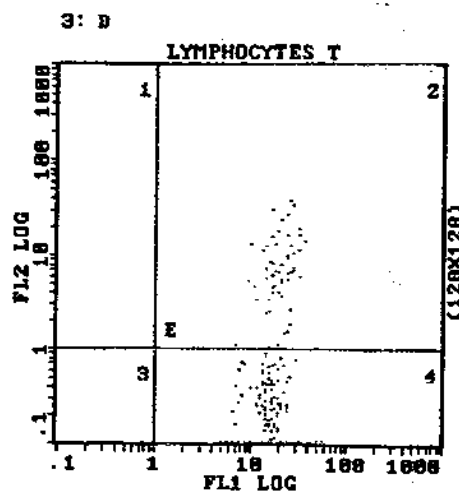
C. Double labelling protocol using the PE conjugated form (Cat. No. 2284) with CD3 FITC (Cat. No. 1281)

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 0.02%.

Example Data

Flow cytometric analysis of a typical double staining experiment CD3 FITC / Vβ3 PE (gating on CD3⁺ lymphocytes).



Quadrant 2: CD3⁺ - Vβ3⁺
 Quadrant 4: CD3⁺ - Vβ3⁻

References

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