

MONOCLONAL ANTIBODY V β 21.3

Cat. No.	Form	Quantity	Presentation
1347	Purified	0.1 mg	Freeze-dried
1483	FITC	50 tests	Liquid 1ml
2025	Biotin	0.1 mg	Freeze-dried
2050	PE	50 tests	Liquid 1 ml

Clone IG125

Isotype IgG2a (mouse)

Immunogen Murine T-cell hybridoma transfected with human V β 21 gene segment

Hybridoma Myeloma NS1xBiozzi spleen cells.

Specificity Human variable β 21 of the T-cell receptor also called TCRBV21S3 according to the nomenclature from Wei et al (1).

This antibody recognizes at least the IGRb01 sequence (2).

This antibody has been further characterized by cell sorting on PBL using this monoclonal antibody, followed by molecular biology analysis of the sorted cells.

Analysis of α chain mRNA using a panel of α specific oligonucleotides shows transcripts for most V α sequences.

Analysis of β chain mRNA by anchored PCR and sequencing, only shows transcripts for IGRb01(10 sequences done).

Many other genes have been classified in V β 21 family. It remains to be determined if the antibody either does not recognize the other V β 21 sequences, or if these sequences are of very low occurrence.

Allelic differences can also exist in this complex subfamily. As IG125 recognizes stable percentage of PBL from many donors, it is not likely that this monoclonal antibody is specific for an allelic form.

IG125 stains from 2.2 to 3.6% of peripheral CD3 positive lymphocytes from 6 healthy adult donors (data on file at Immunotech).

The specificity of this antibody has been confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (3).

Applications

Studies have shown that this V β 21.3 may be useful in T-cell repertoire studies in normal and pathological situations including autoimmune disease particularly rheumatoid arthritis, graft rejection or AIDS.

Superantigenic stimulation of T cells.

May 23, 1996



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.

Recommended Procedures

Flow cytometry

Freeze-dried form: 2 µg/5x10⁵ cells/test.
Liquid form: 20 µl/5x10⁵ cells/test or 100 µl whole blood.

As this antibody recognizes a rare 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. 1347) 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 (Cat No 0819). 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.

B. Double labelling protocol using biotinylated form (Cat. No 2025) with a CD3 FITC (Cat. No. 1281)

1. To 100 µl of whole blood add 10 µl of the reconstituted biotinylated form, and 20 µl of the 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 streptavidine at the usual dilution.

Cat No 1347

- 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 PE conjugated form (Cat No 2050) with a CD3 FITC (Cat No 1281)

- 1 To 100 µl of whole blood add 20 µl of the 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 / Vβ21 3 PE (gating on CD3+ lymphocytes).

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----- Quad Stats -----
File: U3:VB21P12 Sample: VB21.3 PE /3FITC 01 012
Date: 4/ 3/96 Gate G2= R2
Selected Preference: Arithmetic/Linear
Parameters: FL1-H(LOG),FL2-H(LOG) Quad Location: 10.00,13.34
Total= 9535 Gated= 1735

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Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	52	3.00	0.55	176.73	265.57
3 LL	0	0.00	0.00	--	--
4 LR	1683	97.00	17.65	201.58	2.40

Quadrant 2. CD3⁺ - Vβ21⁺
 Quadrant 4. CD3⁺ - Vβ21⁻

