

MONOCLONAL ANTIBODY

V β 16

| Cat. No. | Form | Quantity | Presentation |
|----------|----------|----------|--------------|
| 1559 | Purified | 0.1 mg | Freeze-dried |
| 1560 | FITC | 50 tests | Liquid 1mL |
| 2023 | Biotin | 0.1 mg | Freeze-dried |
| 2294 | PE | 50 tests | Liquid 1 mL |

| | |
|---------------------|---|
| Clone | TAMAYA 1.2 |
| Isotype | IgG1 (mouse) |
| Immunogen | Murine T-cell hybridoma transfected with human V β 16 gene segment. |
| Hybridoma | NS1 x Biozzi spleen cells. |
| Specificity | <p>Human variable β16 chain of the T-cell receptor, also called TCRBV16S1 according to the nomenclature from Wei et al (1).</p> <p>This subfamily contains only one member, corresponding to the published sequence HBP 42 (2), which is identical to HT370 (3). The antibody stains the gene product of these sequences, no crossreactivity with other Vβ subfamilies has been found.</p> <p>TAMAYA 1.2 has been further characterized by cell sorting of PBL followed by molecular biology analysis of the sorted cells. Only Vβ16 sequences were detected (22 sequences analyzed). The Vβ usage was not restricted and all Vα subfamilies were found associated with Vβ16 in the TAMAYA-positive cells.</p> <p>On the average, this antibody stains 0.8% (sd=0.2) of peripheral CD3 positive lymphocytes from 20 healthy adult donors (data on file at Immunotech).</p> <p>TAMAYA 1.2 has been described in Mackensen et al (4).</p> <p>The specificity of this antibody has been confirmed at the First Human TcR Monoclonal Antibody Workshop in San Francisco in 1995 (5).</p> |
| 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 | <p>Freeze-dried forms: 1 mg/mL bovine serum albumin in phosphate-buffered saline</p> <p>Liquid forms: 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.</p> |

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MA003

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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. 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.

Recommended Procedures

Fluorescent microscopy or flow cytometry:

Liquid form: 20 µL / 5 x 10⁵ cells / test or 100 µL whole blood

Freeze-dried form: 2 µg / 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.). 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. 1559) 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 (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 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. 2023) 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 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 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 PE conjugated form (Cat. No. 2294) with CD3 FITC (Cat. No. 1281)

1. To 100 μ L of whole blood add 20 μ L of PE conjugate and 20 μ L of CD3 FITC. Incubate 15 minutes at room temperature.
2. Add 3 mL of PBS/BSA/ NaN_3 . 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/ NaN_3 = PBS/BSA 0.2% / NaN_3 0.02%.

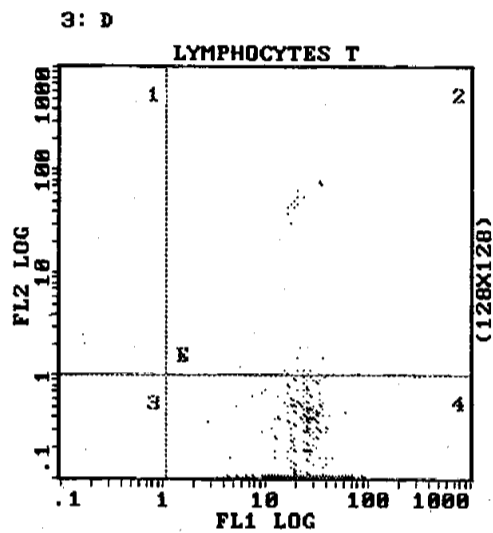
Immunohistochemistry (Cat. No. 1559)

Working dilution: 1:50

Note: this antibody can be used only on frozen tissue sections and cytopins.

Example Data

Flow cytometric analysis of a typical double staining experiment CD3 FITC / V β 16 PE.



Quadrant 2: CD3⁺ - V β 16⁺
 Quadrant 4: CD3⁺ - V β 16⁻

References

- 1) Wei, S., Charnley, P., Robinson, M.A., "The extent of the human germline T-cell receptor V beta gene segment repertoire", 1994, *Immunogenetics*, **40**, 27-36.
- 2) Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debré, 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.
- 3) Plaza, A., Kono, D.H., Theofilopoulos, A.N., "New human V beta genes and polymorphic variants", 1991, *J. Immunol.*, **147**, 4360-4365.
- 4) Mackensen, A., Carcelain, G., Viel, S., Raynal, M.C., Michalaki, H., Triebel, F., Bosq, J., Hercend, T., "Direct evidence to support the immunosurveillance concept in a human regressive melanoma", 1994, *J. Clin. Invest.*, **93**, 1397-1402.
- 5) 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.

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