

MONOCLONAL ANTIBODY V $\delta$ 1

Cat. No.	Form	Quantity	Presentation
1761	Purified	0.1 mg	Freeze-dried
2083	Biotin	0.1 mg	Freeze-dried

<b>Clone</b>	R9.12
<b>Isotype</b>	IgG1 (mouse)
<b>Immunogen</b>	Soluble V $\gamma$ 9 C $\gamma$ /V $\delta$ 1C $\delta$
<b>Hybridoma</b>	X63 AG8.653 x Balb/c spleen cells
<b>Specificity</b>	<p>Human variable <math>\delta</math>1 chain of the T cell receptor. The corresponding sequence is described in (1).</p> <p>This antibody has been characterized on human <math>\gamma/\delta</math> T-cell clones analyzed by PCR. It only stains those clones which express V<math>\delta</math>1 mRNA (2).</p> <p>Sorted V<math>\delta</math>2 and V<math>\delta</math>3 populations are not stained. In all polyclonal <math>\gamma/\delta</math> T-cell lines tested, R9.12 stains a sizeable fraction of cells.</p> <p>In the periphery R9.12 stains from 0.6 to 2.4% of CD3 positive cells from 6 healthy adult donors (data on file at Immunotech).</p> <p>R9.12 antibody reacts only with <math>\gamma\delta^+</math> V<math>\delta</math>1<math>^+</math> cells and not with <math>\alpha\beta^+</math> V<math>\delta</math>1<math>^+</math> cells. V<math>\delta</math>1<math>^+</math> <math>\gamma\delta^+</math> cells are the major T-cell population at birth and are overexpressed in intestinal intraepithelial lymphocytes (3).</p>
<b>Applications</b>	<p>T-cell repertoire studies in normal and in pathological situations, including autoimmune diseases, chronic inflammatory diseases, cancer, bone marrow transplantation, graft rejection. The majority of <math>\gamma/\delta</math> T-cells in most HIV-1- infected individuals express V<math>\delta</math>1 (4).</p> <p>Flow cytometry Immunohistochemistry: this antibody is only suitable on frozen tissue sections.</p>
<b>Buffer</b>	Freeze-dried forms: 1 mg/ml bovine serum albumin in phosphate buffered saline.

June 20, 1996

MA001

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES


**IMMUNOTECH**  
 A COULTER COMPANY

 B.P. 177 - 13276 Marseille Cedex 9 France  
 Tel. (33) 91 17 27 00 - Fax. (33) 91 41 43 58

**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/5x10<sup>5</sup> cells/test.

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 protocol.

A. Double labelling protocol using the freeze-dried unconjugated form (Cat. No.1761) 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/NaN<sub>3</sub>. Centrifuge 5 minutes 1200 rpm, discard supernatant.
3. Add 100 µl of secondary antibody F(ab')<sub>2</sub> goat anti-mouse Ig conjugated to FITC at usual dilution in PBS/BSA NaN<sub>3</sub>. Incubate 15 minutes at room temperature.
4. Repeat step 2 (washing).
5. Resuspend cells in 100 µl of PBS/BSA/NaN<sub>3</sub> 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. 2083) with CD3 PE (Cat. No.1282).

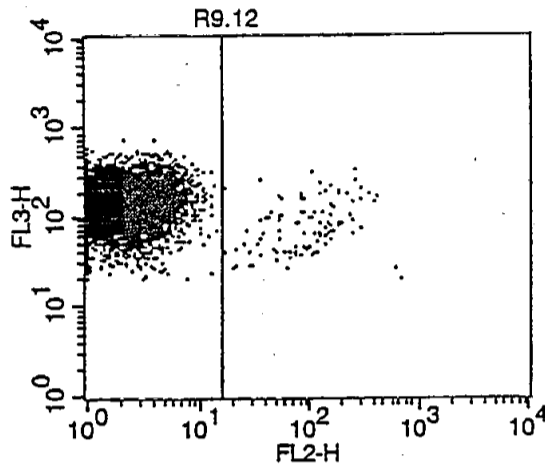
1. To 100 µl of whole blood add 10 µl of the reconstituted biotinylated form, and 20 µl of PE conjugate. Incubate 15 minutes at room temperature.
2. Add 3 ml of PBS/BSA/NaN<sub>3</sub>. Centrifuge 5 minutes at 1200 rpm, discard supernatant
3. Add 100 µl of FITC conjugated streptavidin at the usual recommended dilution.
4. Repeat step 2.
5. Then proceed as usual for lysis of red blood cells and fixing of white cells.

NOTE: PBS/BSA/NaN<sub>3</sub> = PBS/BSA 0.2% / NaN<sub>3</sub> 0.02%.

**Example Data** Flow cytometric analysis of a typical double staining experiment biotinylated Vδ1 / CD3 PE (gating on CD3<sup>+</sup> lymphocytes).

File: R9.12                      Sample ID:  
 Acquisition Date: 13-Jun-96      Gate: G1  
 Gated Events: 8530              Total Events: 54839  
 X Parameter: FL2-H (Log)      Y Parameter: FL3-H (Log)  
 Quad Location: 16, 1

Quad	Events	% Gated	% Total	X Mean	Y Mean
UL	8430	98.83	15.37	2.64	165.82
UR	100	1.17	0.18	127.34	99.36
LL	0	0.00	0.00	***	***
LR	0	0.00	0.00	***	***



Quadrant 1 : CD3<sup>+</sup> - Vδ1<sup>-</sup>

Quadrant 2 : CD3<sup>+</sup> - Vδ1<sup>+</sup>

## References

- 1) Hata S., Clabby M., Devlin P., Spits H., De Vries J.E., Krangel M.S., "Diversity and organization of human T cell receptor δ variable gene segments", 1989, *J. Exp. Med.*, **169**, 41-57
- 2) Romagné, F., Peyrat, M.A., Leget, C., Davodeau, F., Houde, I., Necker, A., Hallet, M.M., Vié, H., Bonneville, M., "Structural analysis of γδ TCR using a novel set of TCR γ and δ chain-specific monoclonal antibodies generated against soluble γδ TCR. Evidence for a specific conformation adopted by the Jδ2 region and for a Vδ1 polymorphism", 1996, *J. Immunol. Methods*, **189**, 25-36.
- 3) Peyrat, M.A., Davodeau, F., Houde, I., Romagné, F., Necker, A., Leget, C., Cervoni, J.P., Cerf-Bensusan, N., Vié, H., Bonneville, M., Hallet, M.M., "Repertoire analysis of human peripheral blood lymphocytes using a human Vδ3 region-specific monoclonal antibody. Characterization of Dual T Cell Receptor (TCR) δ-chain expressors and αβ T cells expressing Vδ3JαCα-encoded TCR chains", 1995, *J. Immunol.*, **155**, 3060-3067.
- 4) Hinz, T., Wesch, D., Friese, K., Reckziegel, A., Arden, B., Kabelitz, D., "T cell receptor γδ repertoire in HIV-1-infected individuals", 1994, *Eur. J. Immunol.*, **24**, 3044-3049.

