

MONOCLONAL ANTIBODY Pan γ/δ

Cat. No.	Form	Quantity	Presentation
1349	Purified	0.1 mg	Freeze-dried
1418	Phycoerythrin	50 tests	Liquid 1ml
1571	FITC	50 tests	Liquid 1ml
1958	PE-Cy5	50 tests	Liquid 1ml

Clone Immu 510

Isotype IgG1 (mouse)

Immunogen Soluble γ/δ T-cell receptor

Hybridoma X63.Ag8.653 x Balb/c spleen cells

Specificity Immu 510 recognizes all the γ/δ T cells regardless the variable genes or junction regions they express as assessed by flow immunofluorescence studies on polyclonal γ/δ T-cell lines as well as γ/δ T-cell clones (1-5). It has been characterized in ref. 1. It recognizes 1.6 to 8.9 % of peripheral CD3 positive lymphocytes from 7 healthy adult donors (data on file at Immunotech S.A.).

This antibody works in Western blot techniques: it recognizes the γ/δ complex under non-reducing conditions and the isolated δ chain on reducing conditions.

This antibody immunoprecipitates the three isoforms of the γ/δ T-cell receptor (2).

It gives also excellent results in immunofluorescence on frozen section (paraffin not tested, 4).

Applications The function of γ/δ T cells remains largely unknown. As this antibody works in all applications (immunofluorescence, western blot, immunoprecipitation, frozen section), it is an ideal tool to study the distribution of γ/δ T cells and their functions.

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.

Conjugates FITC: Fluorescein isothiocyanate conjugated (3 to 7 moles of FITC/mole of IgG).
Excitation wavelength: 488 nm, maximum emission wavelength: 525 nm.
Main emission color: green.
Phycoerythrin (PE): R-Phycoerythrin conjugated (1 mole of phycoerythrin/mole of IgG).
Excitation wavelength: 488 nm, maximum emission wavelength: 575 nm.
Main emission color: orange-red.

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PE-Cy5: The IgG is conjugated to a tandem dye, constituted of R-Phycoerythrin covalently linked to Cyanine 5.

Excitation wavelength: 488 nm, maximum emission wavelength: 670 nm.

Main emission color: deep-red.

Limitation: PE-Cy5 conjugates are recommended for use only on flow cytometers equipped with a 675 nm band pass filter in front of the third fluorescence detector.

The three liquid forms of the antibody are ready-for-use.

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

Flow cytometry:

Freeze-dried form: 2 µg/5x10⁵ cells/test or 10 µl of the reconstituted purified antibody.

Liquid form: 20 µl/5x10⁵ cells/test or 100 µl whole blood.

As this antibody recognizes a rare population, around 3.6% of peripheral blood lymphocytes(4), 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. 1349) with a CD3 PE antibody (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 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 PE conjugated CD3 (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.

Cat. No. 1349

B. Double labelling protocol using conjugated form (Cat. No. 1418, 1571 or 1958) with a CD3 antibody (Cat. No. 1281 or 1282)

1. To 100 µl of whole blood add 20 µl of pan γ/δ FITC, PE or PE-Cy5 conjugate and 20 µl of CD3 FITC or PE 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.

Example Data

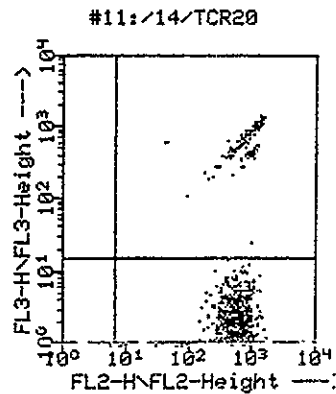
Flow cytometric analysis of a typical double staining experiment Pan γ/δ PE-Cy5, CD3 PE.

#11:/14/TCR20

----- Quad Stats -----

File: #11:/14/TCR20 Sample: 049
 Date: 10/31/95 Gate G5= R5
 Selected Preference: Arithmetic/Linear
 Parameters: FL2-H(LOG),FL3-H(LOG) Quad Location:
 Total= 7933 Gated= 1475

Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	131	8.88	1.65	850.28	643.06
3 LL	0	0.00	0.00	--	--
4 LR	1344	91.12	16.94	591.12	1.88



Quadrant 2: CD3⁺ γ/δ ⁺
 Quadrant 4: CD3⁺ γ/δ ⁻

This analysis has been done with a gating on the CD3 positive population.

References

- 1) Davodeau, F., Houde, I., Bouloï, G., Romagné, F., Necker, A., Canavo, N., Peyrat, M.A., Hallet, M.M., Vié, H., Jacques, Y., Mariuzza, R., Bonneville, M., "Secretion of disulfide-linked human T-cell receptor $\gamma\delta$ heterodimers", 1993, J. Biol. Chem., **268**, 21, 15455-15480.
- 2) Davodeau, F., Peyrat, M.A., Houde, I., Hallet, M.M., de Libero, G., Vié, H., Bonneville, M., "Surface expression of two distinct functional antigen receptors on human $\gamma\delta$ T cells", 1993, Science, **260**, 1800-1802.
- 3) Davodeau, F., Peyrat, M.A., Romagné, F., Necker, A., Hallet, M.A., Vié, H., Bonneville, M., "Dual T cell receptor beta chain expression on human T lymphocytes", 1995, J. Exp. Med., **181**, 1391-1398.
- 4) Peyrat, M.A., Davodeau, F., Houde, I., Romagné, F., Necker, A., Leget, C., Cervoni, J.P., Cerf-Bensoussan, N., Vié, H., Bonneville, M., Hallet, M.M., "Repertoire analysis of human PBL using a human V delta 3 region specific mAb. Characterization of dual TCR delta chain expressors and alpha beta T cells expressing V delta 3/J alpha/C alpha-encoded TCR chains", 1995, J. Immunol., **155**, 3060-3067.
- 5) Thibault, G., Bardos, P., "Compared TCR and CD3 ϵ expression on $\alpha\beta$ and $\gamma\delta$ T cells. Evidence for the association of two TCR heterodimers with three CD3 ϵ chains in the TCR/CD3 complex", 1995, J. Immunol., **154**, 3814-3820.