

MONOCLONAL ANTIBODY TCR Pan α/β

Cat. No.	Form	Quantity	Presentation
1466	Purified	0.1 mg	Freeze-dried
1467	Phycoerythrin	50 tests	Liquid 1 ml
1957	PE-Cy5	50 tests	Liquid 1 ml

Clone BMA 031

Isotype IgG2b (mouse)

Immunogen Human CD2 positive cells

Hybridoma X63 Ag 8.653 x Balb/c spleen cells.

Specificity This antibody recognizes a monomorphic determinant of the human α/β chain of the T-cell receptor complex. It allows the enumeration of α/β^+ T-cell population by flow cytometry (1-4).

It stains 95.1 to 98.4% of peripheral CD3 positive cells from 6 healthy adult donors (data on file at Immunotech S.A.).

It is also suitable for immunohistochemistry only on frozen tissue sections.

Applications T-cell receptor studies in normal and pathological situations. Quantification of TCR α/β^+ cells versus TCR γ/δ^+ cells.

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 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.

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 two liquid forms of the antibody are ready-for-use.

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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

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.

A. Double labelling protocol using freeze-dried unconjugated form (Cat. No. 1466) with a CD3 PE antibody (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 1200 rpm, discard supernatant.
3. Add 100 µl of secondary antibody F(ab')₂ goat anti-mouse Ig conjugated to FITC at its 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.

B. Double labelling protocol using conjugated form (Cat. No. 1467 or 1957) with a CD3 antibody (Cat. No. 1281 or 1282)

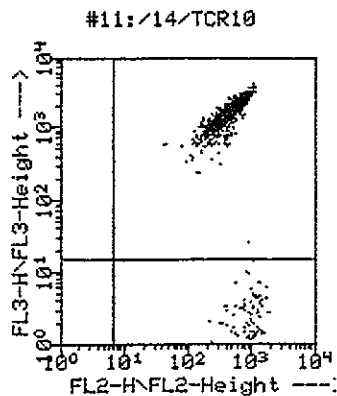
1. To 100 µl of whole blood, add 20 µl of TCR Pan α/β PE or PE-Cy5 conjugate and 20 µl of CD3 FITC or PE conjugate. Incubate 15 minutes at room temperature.
2. Add 3 ml of PBS/BSA/NaN₃. Centrifuge 5 minutes at 1200 rpm, discard supernatant.
3. Proceed as usual for lysis of red blood cells and fixing of white cells.

Example Data

Flow cytometric analysis of a double staining experiment TCR Pan $\alpha\beta$ PE-Cy5/
CD3 PE using recommended procedure.

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

File:	#11:/14/TCR10	Sample:	039		
Date:	10/31/95	Gate:	GS= R5		
Selected Preference:	Arithmetic/Linear				
Parameters:	FL2-H(LOG), FL3-H(LOG)				
Total=	8569	Gated=	1431		
Quad	Events	% Gated	% Total	Xmean	Ymean
1 UL	0	0.00	0.00	--	--
2 UR	1279	89.38	14.93	422.12	1502.42
3 LL	0	0.00	0.00	--	--
4 LR	152	10.62	1.77	931.48	2.21



Quadrant 2: CD3⁺ $\alpha\beta$ ⁺

Quadrant 4: CD3⁺ α/β ⁻

This analysis has been done with a gating on the CD3 positive population to demonstrate that BMA031 only recognizes the CD3⁺ cells expressing TCR $\alpha\beta$ (Quadrant 2), and not the CD3⁺ cells expressing TCR $\gamma\delta$ (Quadrant 4).

References

- 1) Borst, J., Van Dongen, J.J.M., De Vries, E., Comans-Bitter, W.M., Van Tol, M.J.D., Vossen, J.M., Kurrle, R., "BMA031, a monoclonal antibody suited to identify the T-cell receptor alpha beta/CD3 complex on viable human T lymphocytes in normal and disease states", 1990, Human Immunol., **29**, 175-188.
- 2) 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.
- 3) 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.
- 4) 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.

