

MONOCLONAL ANTIBODY

CD4

Cat. No.	Form	Quantity	Presentation
0398	Purified	0.2 mg	Freeze-dried
0704	Biotin	0.2 mg	Freeze-dried
0447	Purified	100 tests	Liquid 2 mL
0448	FITC	100 tests	Liquid 2 mL
0449	PE	100 tests	Liquid 2 mL
1574	PE-Cy5	100 tests	Liquid 2 mL
2468	APC	100 tests	Liquid 1 mL

**Warning** *APC-conjugated forms of the IOTest® line of reagents are to be used at 10 µL / test instead of 20 µL / test.*

**Clone** 13B8.2

**Isotype** IgG1 κ (mouse)

**Immunogen** Human thymocytes

**Hybridoma** NS1 x Balb/c spleen cells

**Specificity** The CD4 antigen is a monomeric transmembrane glycoprotein with a molecular weight (Mr) of 60 kDa. CD4 molecule is expressed on a specific subset of peripheral blood T lymphocytes named "helper / inducer" (1). The CD4 antigen is present on approximately 45% of peripheral blood lymphocytes (T4 lymphocytes), 80% of thymocytes and with a lower density on 100% of monocytes and on some neutrophils (2, 3).

CD4 acts as an accessory molecule to the T cell receptor (TcR) complex during T-cell activation restricted to the Class II major histocompatibility complex (MHC). The CD4 antigen is also known to be one of the human immunodeficiency virus (HIV) receptors (3-5). The T lymphocyte subset that expresses CD4 is involved in T-T, T-B, and B-macrophage cellular interactions (6).

The 13B8.2 monoclonal antibody antagonizes HIV effects *in vitro*.

The 13B8.2 monoclonal antibody has been assigned to the CD4 cluster of differentiation at the 3rd International Workshop in Boston (1986) (7).

**Applications** Flow cytometry.  
Studies of CD4 expressing 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.

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## Conjugation

- FITC:** Fluorescein isothiocyanate (FITC) is conjugated at 4-7 moles of FITC per mole of IgG.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 525 nm  
Main emission color: Green
- PE:** R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per 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 cyanin 5.1 at 0.7-1 mole of PE-Cy5 per mole of IgG.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 670 nm  
Main emission color: Deep-red
- APC:** Allophycocyanin (APC) is conjugated at 0.7-1 mole of APC per mole of IgG.  
Excitation wavelength: 633-635 nm  
Maximum emission wavelength: 660 nm  
Main emission color: Deep-red

**Limitation:** APC conjugates are recommended for use only on flow cytometers equipped with an exciting source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

## Reconstitution and Storage

The freeze-dried forms should be stored at 2-8°C until the expiration date stated on the vial label. Reconstitute with 1 mL of distilled water. No preservative has been added. The reconstituted forms should be stored at -20°C until the expiration date stated on the vial label. 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 forms for up to one month at 2-8°C

The purified liquid form should be stored at 2-8°C until the expiration date stated on the vial label

The conjugated forms should not be frozen and should be stored in the dark at 2-8°C until the expiration date stated on the vial label.

## Recommended Procedures

### Flow cytometry

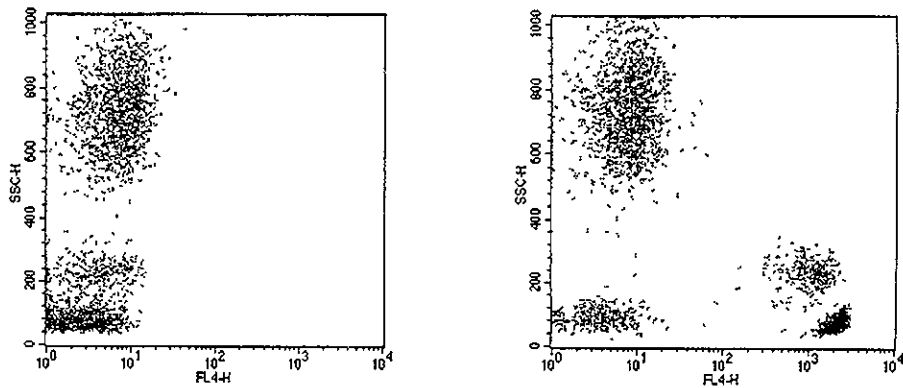
Purified, FITC-, PE- and PE-Cy5-conjugated forms. 20 µL /  $5 \times 10^5$  cells or 100 µL of whole blood.

**APC-conjugated form:** A specific calibration is applied to facilitate the blending of conjugated antibodies in multiparametric flow cytometry.  
10 µL /  $5 \times 10^5$  cells or 100 µL of whole blood.

**Limitation:** R-phycoerythrin (PE) is sensitive to light exposure. Consequently, PE- or PE-Cy5-conjugated antibodies are not suitable for fluorescence microscopy.

## Results Example

The graphs below are double parameter representations (Side Scatter *versus* Fluorescence 4) of a lyzed whole blood sample from an healthy donor. Staining is with IgG1-APC (left) and CD4-APC (right). Along the Y axis, lymphocytes are events with low side scatter values, monocytes show low to medium side scatter values and neutrophils show medium to high side scatter values.



Isotypic control, IgG1-APC (Cat No 2475)      Specific staining, CD4-APC (Cat No 2468)

Analysis is with a Becton Dickinson FACSCalibur™ flow cytometer equipped with CELLQuest™ software.

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 FACSCalibur and CELLQuest are trademarks of Becton Dickinson Immunocytometry Systems (BDIS)

## References

- 1) Sprent, J, "T lymphocytes and the thymus", 1989, in Fundamental Immunology, 2nd edition, Paul, W.E., Ed., Raven Press, p 69-93.
- 2) Hannet, I, Erkeller-Yuksel, F., Lydyard, P, Deneys, V., DeBruyère, M., "Developmental and maturational changes in human blood lymphocyte subpopulations", 1992, Immunol. Today, **13**, 6, 215-218.
- 3) Micelli, M C , Pames, J.R., "The role of CD4 and CD8 in T cell activation", 1991, Sem. Immunol., **3**, 133.
- 4) Centers for Disease Control and Prevention: 1994 revised guidelines for performance of CD4<sup>+</sup> T-cells determinations in persons with human immunodeficiency virus (HIV) infection, 1994, Morbidity and Mortality Weekly Report, **43**, 3.
- 5) Fauci, A.S , "The human immunodeficiency virus: infectivity and mechanism of pathogenesis", 1988, Sciences, **239**, 617
- 6) van Agthoven, A., Terhorst, C., Reinherz, E.L., Schlossman, S.F., 1991, "Characterization of T cell surface glycoproteins T1 and T3 present on all human peripheral T lymphocytes and functional mature T lymphocytes", Eur. J Immunol **1981**, 11-18.
- 7) Taylor, G.M., Williams, A , Morten, J., Morten, H. "Analysis of CD4 monoclonal antibodies using human X mouse hybrid cell-lines OKT4", 1987, in Leukocyte Typing III, White Cell Differentiation Antigens, McMichael, A.J , et al, Eds., Oxford Univ. Press, 1987, p. 234-238.

