

MONOCLONAL ANTIBODY

CD45

Cat. No.	Form	Quantity	Presentation
0781	Purified	100 tests	Liquid 2 mL
0782	FITC	100 tests	Liquid 2 mL
2077	PE-Cy5	100 tests	Liquid 2 mL
2078	PE	100 tests	Liquid 2 mL
2473	APC	100 tests	Liquid 1 mL

Warning *APC-conjugated forms of the IC_{Test}[®] line of reagents are to be used at 10 µL / test instead of 20 µL / test.*

Clone J33

Isotype IgG1 (mouse)

Immunogen Laz 221 ALL cell line

Hybridoma NS1 x Balb/c spleen cells

Specificity The CD45 family of transmembrane glycoproteins is comprised of five different isoforms with molecular weights of 180 to 220 kDa.

The different isoforms are generated by alternative splicing of three exons encoding peptide segments designated A, B and C. All the isoforms have the same eight amino-terminal amino acids, which are followed by the various combinations of A, B and C peptides. The remaining, membrane-proximal portion of all the CD45 isoforms are the same (1).

CD45 is expressed on the surface of all human leucocytes and plays an important regulatory role in T-cell activation.

The J33 monoclonal antibody binds to all the CD45 isoforms present on human leucocytes.

The J33 antibody has been assigned to the CD45 cluster of differentiation at the 3rd International Workshop on Human Leucocyte Differentiation Antigens in Boston (1986).

Applications Flow cytometry

Buffer 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 5-8 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 excitation source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

Storage

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 / 5×10^5 cells or 100 µL whole blood.

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

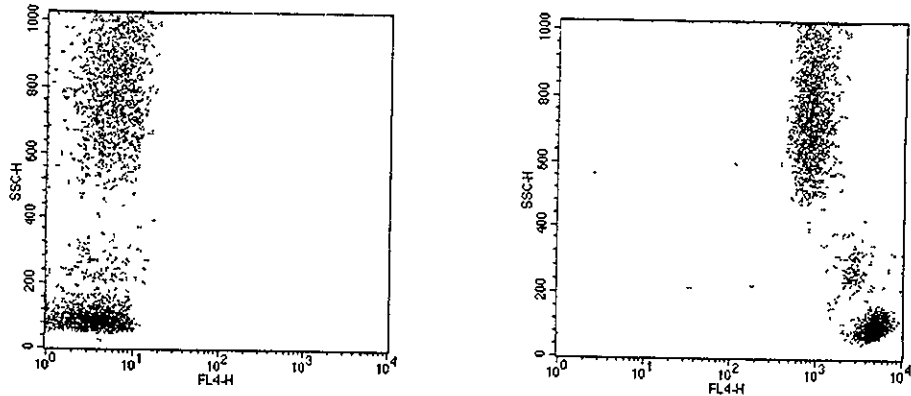
Limitation: R-phycoerythrin (PE) is sensitive to light exposure. Consequently, PE- or PE-Cy5-conjugated antibodies are not suitable for fluorescence microscopy.

Cat No. 0781

Results

The dot-plots below are double parameter representations (Side scatter lin *versus* Fluorescence 4 log) of IgG1-APC- and CD45-APC-labeled leucocytes of lyzed whole blood samples of the same healthy donor.

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, CD45-APC (Cat No 2473)

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) Serra-Pages, C, Monimoto, C., Schlossman, S.F., Saito, H, Streuli, M., "Characterization of CD45 mAb", 1995, in Leucocyte Typing V, White cell differentiation antigens, Schlossman, S.F, et al., Eds., Oxford Univ. Press, p. 389-391.
- 2) Cobbold, S., Hale, G., Waldmann, H., "Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters", 1989, in Leucocyte Typing III, White cell differentiation antigens, Knapp, W., et al., Eds., Oxford Univ. Press, p. 796-803
- 3) Pesando, J.M, Hoffman, P., Abed, M., "Antibody-induced antigenic modulation is antigen dependent: characterization of 22 proteins on a malignant human B cell line", 1986, J. Immunol., **137**, 3689-3695.