

PN IM2644**CD20 - PC5****(B9E9)****100 tests
10 µL/test****IO Test[®]**
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

The CD20 molecule is a nonglycosylated membrane-embedded protein which exhibits an hydrophobic region spanning four times the membrane. The long N- and C-terminal ends of the molecule are located within the cytoplasm. The level of phosphorylation of the cytoplasmic tail is responsive for the heterogeneity of the molecular weight ranging from 33 to 37 kDa (1). The CD20 may also exist on the cell surface as a homo-oligomeric complex forming with other molecules a multimeric receptor complex (2).

The expression of CD20 is restricted on B-lineage cells. Its expression occurs early in pre-B lymphocyte development, persists in B-lymphocyte ontogeny and is lost upon ultimately plasma cells differentiation defining thus CD20 as a B-cell marker (2,3). The CD20 molecule is present on all B lymphocytes from peripheral blood, lymph node, spleen, tonsil and bone marrow. The CD20 antigen may be weakly expressed on a subset of resting T lymphocytes (4,5). These CD20dim T lymphocyte subset represents 2-3% of peripheral blood lymphocytes and exhibit CD8, TcR γ/δ and CD45RO pattern expression (5). CD20 antigen is not expressed on other leucocyte subsets including, NK cells, monocytes and granulocytes.

The CD20 antigen is involved in the regulation of B-lymphocytes activation and proliferation by regulating transmembrane Ca²⁺ flux. Furthermore, the structure of the CD20 molecule, with a proposed multiple membrane spanning regions, is similar to that of an ion channel (2,4). The CD20 antigen is heavily phosphorylated on activated B-lymphocytes (2,4,1).

In vitro studies have shown that CD20 expression is down-regulated by IL-4, and CD40 molecule (via anti-CD40 antibody) may reverse this inhibition (6).

B9E9 (HRC20) monoclonal antibody was assigned to the CD20 cluster of differentiation at the Vth International Workshop of Human Leucocytes Differentiation Antigen in 1993 (Boston) (2).

REAGENT

Clone B9E9
Isotype IgG2a mouse
Immunogen B cells
Hybridoma P3-X63-Ag 8 653 x Balb/c spleen cells
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation PC5 The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanin 5.1 at 0.7-1 mole of PC5 per mole of IgG
Excitation wavelength: 488 nm
Maximum emission wavelength: 670 nm
Main emission color: Deep-red
Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide

APPLICATION

Flow cytometry
 Studies have shown that CD20 is hyperphosphorylated in hairy cells (7).

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits

in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.

2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.

3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.

4. Do not use antibody beyond the expiration date on the label.

5. Do not expose reagents to strong light during storage or incubation.

6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 °C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25 °C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.

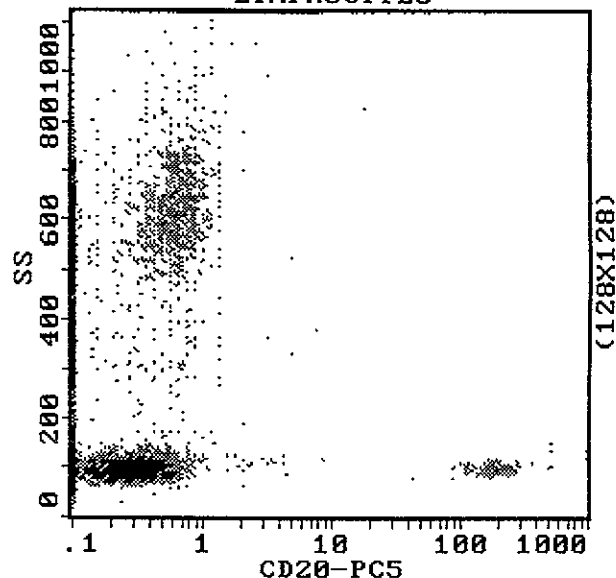
Assay volume: 10 µL/5 x 10⁵ cells / test or 100µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The histograms below are biparametric representations (Side Scatter versus Fluorescence Intensity) of lysed normal whole blood sample. Staining is with CD20-PC5 monoclonal antibody (PN IM2644) gated on leucocytes. The isotypic control labeling is not shown.

Acquisition is with a COULTER R EPICS R XL TM flow cytometer
 Analysis is with the XL System II TM software

LYMPHOCYTES**COULTER**

PARTNERS IN CELL ANALYSIS

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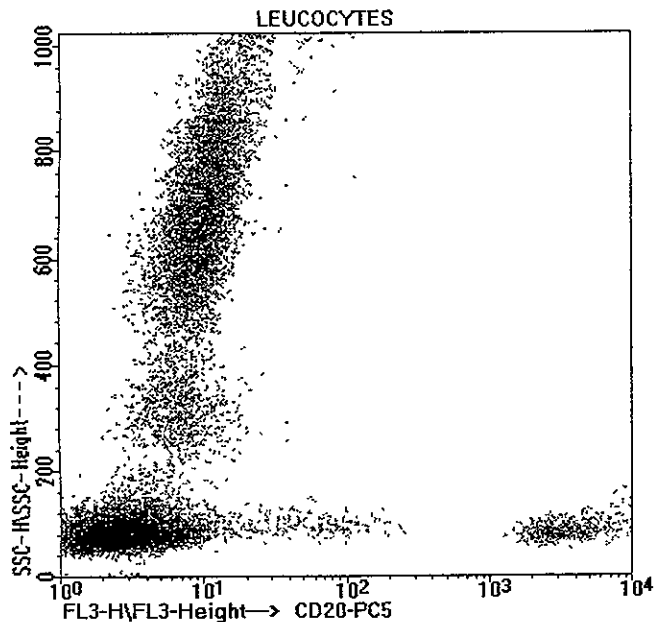
**IMMUNOTECH**
A COULTER COMPANY

PN IM2644 CD20 - PC5 (B9E9)

100 tests
10 µL/test

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Acquisition is with a Becton Dickinson FACScan TM flow cytometer
Analysis is with the LYSYS II TM software



SELECTED RESEARCH REFERENCES

- 1-[852] Tedder, T.F., Engel, P., "CD20 a regulator of cell-cycle progression of B lymphocytes", 1994, Immunol Today, 9, 15, 450-454.
- 2-[49] Zhou, L.J., Tedder, T.F., "CD20 Workshop panel report", 1995, Leucocyte Typing V, White Cell Differentiation Antigens Schlossman, S.F., et al., Eds., Oxford University Press, p. 511-514
- 3-[12] Uckun, F.M., "Regulation of human B-cell ontogeny", 1990, Blood, 10, 76, 1908-1923.
- 4-[1974] Chang, K.L., Arber, D.A., Weiss, L.M., "CD20. a review", 1996, Applied Immunohistochemistry, 1, 4, 1-15.
- 5-[3897] Hultin, L.E., Hausner, M.A., Hultin, P.M., Giorgi, J.V., "CD20 (pan-B cell) antigen is expressed at a low level on a subpopulation of human T lymphocytes", 1993, Cytometry, 14, 2, 196-204
- 6-[534] Dancescu, M., Wu, C., Rubio, M., Delespesse, G., Sarfati, M., "IL-4 induces conformational change of CD20 antigen via a protein kinase C-independent pathway", 1992, J Immunol, 8, 148, 2411-2416
- 7-[2] Sprent, J., "T lymphocytes and the thymus", 1989, Fundamental Immunology, Chap 4, 2nd Ed., 69-93

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