

PN IM3634 CD20-APC
100 tests (B9E9 (HRC20))
10 µL / test



IOTest[®]
 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). This CD20^{dim} 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 Ca^{2+} 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 (1, 2, 4).

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 5th International Workshop of Human Leucocyte Differentiation Antigens in Boston, U.S.A., in 1993 (2).

REAGENT

Clone B9E9 (HRC20)
Isotype IgG2a, mouse
Immunogen B cells
Hybridoma P3-X63-Ag.8.653 x Balb/c
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation Allophycocyanin (APC) is conjugated at 0.5 - 1.5 moles of APC per mole of Ig.
 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).

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

B-cell enumeration by flow cytometry. Study of B-lineage acute lymphoblastic leukemia (ALL) (7). Studies have shown that CD20 is hyperphosphorylated in hairy cells (8).

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 18 - 25°C prior to use.

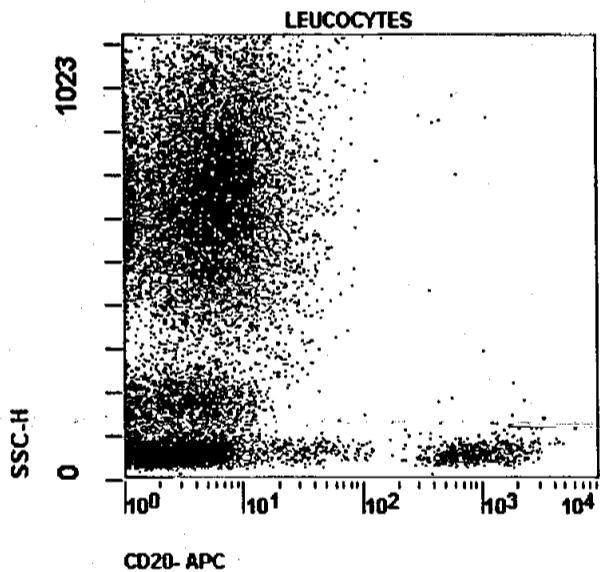
PROCEDURE

This reagent is designed for Flow Cytometry.
 Assay volume: 10 µL per 5×10^5 cells in one test, or per 100 µL whole blood.
 A wash is required to yield optimal results.

EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of a lysed normal whole blood sample. All leucocytes are shown. Staining is with CD20-APC monoclonal antibody (PN IM3635). Isotypic control labeling (PN IM2475) is not shown.

Acquisition is with a BD Biosciences FACSCalibur™ flow cytometer equipped with CELLQuest™ software. Analysis is with EXPO 32™ Cytometer software.



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PARTNERS IN CELL ANALYSIS



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SELECTED RESEARCH REFERENCES

1. Tedder, T.F., Engel, P., "CD20: A regulator of cell-cycle progression of B lymphocytes", 1994, *Immunol. Today*, 9, 15, 450-454.
2. 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, 511-514.
3. Uckun, F.M., "Regulation of human B-cell ontogeny", 1990, *Blood*, 10, 76, 1908-1923.
4. Chang, K.L., Arber, D.A., Weiss, L.M., "CD20: A review", 1996, *Appl. Immunohistochem.*, 1, 4, 1-15.
5. 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*, 2, 14, 196-204.
6. 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. Jennings, C.D., Foon, K.A., "Recent advances in flow cytometry : application to the diagnosis of hematologic malignancy", 1997, *Blood*, 8, 90, 2863-2892.
8. Sprent, J., "T lymphocytes and the thymus", 1989, *Fundamental Immunology*, Chap 4, 2nd Ed., 69-93.

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