

**Analyte Specific Reagent.**

Analytical and performance characteristics are not established.

**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 responsible 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 heavy on B-lineage cells. It appears early in pre-B lymphocyte development, persists throughout B-lymphocyte ontogeny and is lost upon plasma cell differentiation (2, 3). Thus, the CD20 molecule is present on all B lymphocytes whatever the hematopoietic tissue where they are found (peripheral blood, lymph nodes, spleen, tonsil, or bone marrow). Apart from B cells, the CD20 antigen may be weakly expressed on a subset of resting T lymphocytes (4, 5). However, CD20 is not expressed on other leucocyte subsets including NK cells, monocytes and granulocytes.

The B9E9 (HRC20) monoclonal antibody has been assigned to the CD20 cluster of differentiation at the 5th International Workshop on Human Leucocyte Differentiation Antigens in Boston, USA, in 1993 (2).

**REAGENT**

IOTest CD20-ECD Conjugated Antibody

PN IM3607U – 1 mL Liquid – 10 µL / test\*.

<b>Clone</b>	B9E9 (HRC20)
<b>Isotype</b>	Mouse IgG2a
<b>Immunogen</b>	B cells
<b>Hybridoma</b>	P3-X63-Ag.8.653 x Balb/c
<b>Source</b>	Ascites fluid
<b>Purification</b>	Ion exchange or affinity chromatography
<b>Conjugation</b>	Energy Coupled Dye (ECD): The Ig is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to Texas Red at 0.8-1 mole of ECD per mole of Ig.

**Fluorescence** ECD (Red)

Excites at 486–580 nm  
Emits at 610–635 nm

**Buffer**

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

**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.
7. Use good laboratory practices when handling this reagent.

**STORAGE CONDITIONS AND STABILITY**

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

**EVIDENCE OF DETERIORATION**

Any change in the physical appearance of this ECD-labeled reagent (clear, colorless to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

**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.

**SELECTED RESEARCH REFERENCES**

1. Tedder, T.F., Engel, P., "CD20: a regulator of cell-cycle progression of B lymphocytes", 1994, Immunol. Today, 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, 76, 1908-1923.
4. Chang, K.L., Arber, D.A., Weiss, L.M., "CD20: a review", 1996, Appl. Immunohistochem., 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, 14, 196-204.

**PRODUCT AVAILABILITY**

IOTest CD20-ECD Conjugated Antibodies  
PN IM3607U – 1 mL Liquid – 10 µL / test\*.

ECD is licensed under patent 4,520,104.

For additional information in the USA, call 800-526-7694.

Outside the USA, contact your local Beckman Coulter representative.

[www.beckmancoulter.com](http://www.beckmancoulter.com)

**TRADEMARKS**

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Manufactured by:  
Immunotech, a Beckman Coulter Company  
130, avenue de Lattre de Tassigny, B.P. 177  
13276 Marseille Cedex 9, France

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(\*): 10 µL is the quantity of product sufficient to stain 5 x 10<sup>5</sup> cells in a standard immunofluorescence assay

