

For Research Use Only. Not For Use In Diagnostic Procedures.

### SPECIFICITY

The CD19 antigen is a single chain, type I integral membrane glycoprotein with a molecular weight of 95 kDa (1). Its extracellular domain comprises 280 amino acids organized as two C2-type Ig-like domains separated by a smaller potentially disulfide-linked domain. The extensive cytoplasmic domain of CD19 contains nine conserved tyrosine residues, and several of these are located within potential src-homology region 2 (SH2)-binding sites. The CD19 molecule shows a full B lineage expression, from early pre-B cells to mature B lymphocytes. Its expression is lost during maturation to plasma cells (2 – 4). The CD19 antigen is also expressed on the membrane of follicular dendritic cells and on most stabilized B cell lines.

Concerning the percentage of CD19 expressing cells within peripheral blood lymphocytes, in healthy adult donors, reports give spread range of values from 6.4% +/- 2.9% (5) to 13.2% +/- 4.9% (6). The percent of CD19<sup>+</sup> lymphocytes is higher (about 16.1%) during early childhood (< 5 years) and decreases progressively to the adult values (5). CD19 expression is not observed in normal T lymphocytes, NK cells, monocytes, and granulocytes (2). Quantitative determination of surface antibody binding capacities (ABC) of CD19 on B lymphocytes from healthy adult donors gave a range of 11,689 to 25,252 ABC per cell (6).

CD19 is a signal transduction molecule that regulates lymphocyte development, activation, and differentiation (7). CD19 can be associated within the membrane to form hetero-oligomeric structures with other surface molecules including CD21, the complement receptor type 2 (CR2), and CD81 (TAPA-1) (8). The extracellular and transmembrane domains of CD19 are required for the interaction of this molecule with CD21 and CD81. Co-ligation of the CD19-CD21-CD81 complex with the surface IgM-B-cell antigen receptor (BCR) leads to the phosphorylation of CD19 by Syk (9), followed by the recruitment, through tyrosine phosphorylated CD19, of positive signal transduction effectors such as phosphatidylinositol 3-kinase (PI 3-kinase), Lyn, and Fyn (10, 11).

*In vitro* studies show that the J4.119 monoclonal antibody (mAb) blocks labelled B4 mAb binding on Raji cells (1). J4.119 cross reacts with B cells from *Macaca fascicularis*, *Macaca mulatta* and *Papio cynocephalus* (12). It reacts weakly with B cells from Rhesus monkey (13). The J4.119 mAb was assigned to the CD19 cluster of differentiation at the 4<sup>th</sup> International Workshop on Human Leucocyte Differentiation Antigens in Vienna, Austria (1989) (1).

### REAGENT

IOTest CD19-PC7 Conjugated Antibodies  
PN IM3628 - 100 tests - 10 µL/test

**Clone** J4.119  
**Isotype** IgG1 mouse  
**Immunogen** SKLY 18 lymphoma cells  
**Hybridoma** NS1 x Balb/c spleen cells  
**Source** Ascites Fluid  
**Purification** Ion exchange or affinity chromatography  
**Conjugation** PC7: the IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanine 7 (indocarbocyanine) at 0.5 – 1.5 mole of PC7 per mole of Ig.

Excitation wavelength: 488 nm  
Emission wavelength range: 750 – 810 nm  
Main emission color: Far red

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

### APPLICATION

Flow cytometry:  
Studies of CD19-expressing lymphocytes.

### 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 expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.

### 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.

### 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.

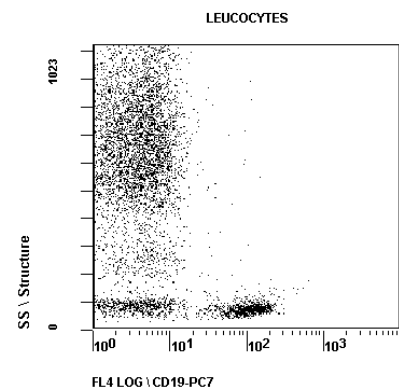
### PROCEDURE

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

### EXAMPLE DATA

The graph below is a biparametric representation (Side Scatter *versus* Fluorescence Intensity) of lysed normal whole blood sample. Staining is with CD19-PC7 monoclonal antibody (PN IM3628). Gate is on leucocytes.

Acquisition is with a COULTER<sup>®</sup> EPICS<sup>®</sup> XL<sup>™</sup> flow cytometer. Analysis is with EXPO<sup>™</sup> analysis software (Beckman Coulter PN 6605433).



### SELECTED RESEARCH REFERENCES

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### PRODUCT AVAILABILITY

IOTest CD19-PC7 Conjugated Antibodies  
PN IM3628 - 100 tests - 10 µL/test  
PE is licensed under patent 4,520,110

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

Outside the USA, contact your local  
Beckman Coulter representative.

### TRADEMARKS

IOTest is a registered trademark of  
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