

**Analyte Specific Reagent.**

**Analytical and performance characteristics are not established.**

**SPECIFICITY**

The CD45 molecule regroups single type I transmembrane glycoproteins with a molecular weight (Mr) ranging from 180 to 220 kDa (1, 2).

The CD45 proteins are all coded by a single gene composed of 33 exons (1). Differential splicing of exons 4, 5 and 6 (which encode A, B, and C determinant respectively) generates at least five isoforms of the CD45 protein (i. e. ABC, AB, BC, B and 0) identified by relevant antibodies (3). Antibodies reactive with all five isoforms are clustered as CD45 (CD45 "non-restricted" or pan-CD45). Antibodies reactive with restricted epitope are clustered as CD45R. CD45RA, CD45RB, CD45RC antibodies recognize isoforms which include the expression of A, B and C exon respectively.

The CD45 protein is composed by a large cytoplasmic region with two tyrosine phosphatase domains. The extracellular region distal to the membrane represented by A, B and C determinants contains potential sites for O-linked glycosylation. The extracellular region proximal to the membrane is probably constituted by three fibronectin type III domains with numerous N-linked carbohydrate sites (4, 3). Alternative splicing and glycosylation are responsible for the Mr heterogeneity of the molecule.

CD45RA isoforms are expressed on the surface of B and NK lymphocytes as well as on a sub-population of T cells often qualified as being naïve and / or at rest (5).

The CD45RA antigen is present on approximately 50% of CD4<sup>+</sup> T cells and on approximately 75% of CD8<sup>+</sup> T cells (5). CD45RA and CD45RO were the first markers to discriminate naïve T cells (generally speaking CD45RA<sup>+</sup>CD45RO<sup>-</sup>) from memory T cells (generally speaking CD45RA<sup>-</sup>CD45RO<sup>+</sup>) (6). The density of expression of CD45RA isoforms declines during the *in vitro* activation of T cells, whilst expression of the CD45RO isoform continues to increase. More recent studies suggest however that certain CD8<sup>+</sup> memory T cells can go backwards in the direction of a CD45RA<sup>+</sup> phenotype.

Monocytes and dendritic cells express predominantly low molecular weight isoform (i. e. CD45R0, CD45RB) with a subset expressing CD45RA and CD45RC. Granulocytes principally express only the lower molecular weight isoform (i. e. CD45R0, CD45RB) (3).

The cytoplasmic protein tyrosine phosphatase activity (PTPase) of the CD45 molecule may influence the function of many other receptor pathways by dephosphorylation of intracellular signaling molecules (7, 3).

The ALB11 monoclonal antibody (mAb) reacts with significant portions of peripheral CD4<sup>+</sup> T cells, of peripheral CD8<sup>+</sup> T lymphocytes, of B lymphocytes and of monocytes. The coexpression of CD4 and CD45RA allows the identification of the suppressor-inducer subpopulation of CD4 lymphocytes.

The ALB11 mAb has been assigned to the CD45RA cluster of differentiation at the fifth International Workshop on Human Leucocyte Differentiation Antigens held in Boston, USA, in 1993 (8).

**REAGENT**

IOTest CD45RA-PE Conjugated Antibody  
PN IM1834U – 2 mL Liquid – 20 µL / test\*.

<b>Clone</b>	ALB11
<b>Isotype</b>	IgG1, mouse
<b>Immunogen</b>	Human T-ALL cells
<b>Hybridoma</b>	NS1 x Balb/c spleen cells
<b>Source</b>	Ascites fluid
<b>Purification</b>	Ion exchange or affinity chromatography
<b>Conjugation</b>	R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.
<b>Fluorescence</b>	PE (orange-red) Excites at 486 – 580 nm Emits at 568 – 590 nm

**REAGENT CONTENTS**

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide (NaN<sub>3</sub>) as preservative, and 2.0 mg / mL bovine serum albumin (BSA).

**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. Do not use antibody beyond the expiration date on the label.
3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
5. Minimize exposure of reagent to 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 PE-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 preparation 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. Weiss, L.M., Arber, D.A., Chang, K.L., "CD45 : a review", 1993, Applied Immunohistochemistry, 1, 166-181.
2. J Poppema, S., Lai, R., Visser, L., Yan, X.J., "CD45 (Leucocyte Common Antigen) expression in T and B lymphocyte subsets", 1996, Leukemia and Lymphoma, 20, 217-222.
3. Sewell, W.A., Cooley, M.A., Hegen, M., "CD45 Workshop Panel Report", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens, 499-502.
4. Okumura, M., Thomas, M.L., "Regulation of immune function by protein tyrosine phosphatases", 1995, Cur. Opin. Immunol., 7, 312-319.
5. Morimoto, C., Letvin, N.L., Distaso, J.A., "The isolation and characterization of the human suppressor inducer T cell subset", 1985, J. Immunol., 134, 1508-1515.
6. Faint, J.M., Annels, N.E., Curnow, S.J., Shields, P., Pilling, D., Hislop, A.D., Wu, L., Akbar, A.N., Buckley, C.D., Moss, P.A.H., Adams, D.H., Rickinson, A.B., Salmon, M. "Memory T cells constitute a subset of the human CD8<sup>+</sup> CD45RA<sup>+</sup> pool with distinct phenotypic and migratory characteristics", 2001, J. Immunol., 167, 212-220.
7. Neel, B.G., "Role of phosphatases in lymphocyte activation", 1997, Cur. Opin. Immunol., 9, 405-420.
8. Macardle, P.J., Flego, L., Khouri, H., Zola, H., "Classification of CD45 mAb according to the susceptibility of the CD45 isoforms to cleavage by papain", 1995, Leucocyte Typing V, White Cell Differentiation Antigens, Schlossman, S.F., et al., Eds., Oxford University Press, p 391-393.

(\*) : 20 µL is the quantity of product sufficient to stain

5 x 10<sup>5</sup> cells in a standard immunofluorescence assay



# IOTest<sup>®</sup> CD45RA-PE

PN IM1834U – 2 mL Liquid – 20 µL / test\* – Clone ALB11

## PRODUCT AVAILABILITY

IOTest CD45RA-PE Conjugated Antibody  
PN IM1834U – 2 mL Liquid – 20 µL / test\*.

PE is licensed under patent 4,520,110.

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5 x 10<sup>5</sup> cells in a standard immunofluorescence assay

