

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. CD45R0 antibodies react with CD45 isoforms lacking the exon A-, B- and C-encoded regions (1).

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⁺ T cells and on approximately 75% of CD8⁺ T cells (5). CD45RA and CD45R0 were the first markers to discriminate naïve T cells (generally speaking CD45RA⁺CD45R0⁻) from memory T cells (generally speaking CD45RA⁻CD45R0⁺) (6).

The density of expression of CD45RA isoforms declines during the *in vitro* activation of T cells, whilst expression of the CD45R0 isoform continues to increase. More recent studies suggest however that certain CD8⁺ memory T cells can go backwards in the direction of a CD45RA⁺ 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).

2H4LDH11LDB9 (2H4) monoclonal antibody was evaluated during the 3rd and the 4th HLDA workshop on Human Leukocyte Differentiation in Oxford (1986) and Vienna (1989) respectively (8). 2H4LDH11LDB9 (2H4) monoclonal antibody is restricted to the CD45RA antigen (9, 10).

REAGENT

IOTest CD45RA-APC-Alexa Fluor 750
Conjugated antibody
PN A86050 - 50 tests - Liquid - 10 µL/test*

Clone	2H4
Isotype	IgG1, Mouse
Immunogen	T lymphocyte derived from Aotus trivirgatus
Hybridoma Source	NS1 x spleen B cells Ascites fluid
Purification	Affinity chromatography
Conjugation	Allophycocyanin -Alexa Fluor 750 (APC-Alexa Fluor 750) APC-Alexa Fluor 750 / Ig : 0.5 - 1.5
Molar Ratio	
Fluorescence	Excites at 633/638 nm Emits at 775 nm

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.

STATEMENTS OF WARNING

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 considered potentially infectious 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.

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.

PRECAUTIONS

Due to the tandem structure of the fluorochrome, APC-Alexa Fluor 750 also emits light at 660 nm. This secondary emission peak varies from lot-to-lot of APC-Alexa Fluor 750. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a APC-Alexa Fluor 750-conjugate.

(*): 10 µL is the quantity of product sufficient to stain 5 x 10⁵ cells in a standard immunofluorescence assay



SELECTED RESEARCH REFERENCES

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