

Analyte Specific Reagent.

Analytical and performance characteristics are not established.

SPECIFICITY

The CD45 family of molecules regroups single type I transmembrane glycoproteins with molecular weights ranging from 180 to 220 kDa (1).

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 determinants respectively) generates at least five isoforms of the CD45 protein (i.e. ABC, AB, BC, B and O) identified by relevant antibodies (2). 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. The CD45RO antibodies recognize the isoform which lacks the expression of exons A, B and C (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 (2, 3). CD45 is expressed on the surface of all nucleated hematopoietic cells (2). Mixed expression of restricted forms of CD45 among human peripheral T lymphocytes define naive (virgin or resting) CD45RA-positive lymphocytes and memory (primed or activated) CD45RO-positive cells (4). Furthermore, the percentage of CD45RO-positive cells increases with aging (5). CD45RO is weakly expressed on monocytes and granulocytes (2). The UCHL1 monoclonal antibody (mAb) recognizes the 180 kDa isoform of the CD45 which corresponds to the CD45RO restricted form (6, 7). UCHL1 mAb was assigned to the CD45RO cluster of differentiation at the IVth International Workshop on Human Leucocyte Differentiation Antigens in Vienna, Austria, in 1989 (6).

REAGENT

IOTest CD45RO-FITC Conjugated Antibody
PN IM1247U – 2 mL Liquid – 20 µL / test*.

Clone	UCHL1
Isotype	IgG2a, mouse
Immunogen	Human IL-2 dependent T-cell line
Hybridoma Source	X63-Ag.8.653 x Balb/c Ascites fluid
Purification	Ion exchange or affinity chromatography

Conjugation FITC (Fluorescein isothiocyanate) is conjugated at 2 – 5 moles of FITC per mole of Ig.

Fluorescence FITC (Green)
Excites at 468 – 509 nm
Emits at 504 – 541 nm

REAGENT CONTENTS

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide (NaN₃) 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 FITC-labeled reagent (clear, colorless to yellowish-green 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, Appl. Immunohistochem., 1, 166-181.
2. Sewell, W.A., Cooley, M.A., Hegen, M., "CD45 Workshop Panel Report", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens. Kishimoto, T., et al, Eds., Garland Publishing, Inc., 499-502.
3. Okumura, M., Thomas, M.L., "Regulation of immune function by protein tyrosine phosphatases", 1995, Curr. Opin. Immunol., 7, 312-319.
4. Callard, R.E., Gearing, A.J.H., "The cytokines and their receptors: Interleukins IL-2", 1994, The Cytokine FactsBook, Academic Press, 39-45.
5. Poppema, S., Lai, R., Visser, L., Yan, X.J., "CD45 (Leucocyte Common Antigen) expression in T and B lymphocyte subsets", 1996, Leuk. Lymphoma, 20, 217-222.
6. Schwinzer, R., "Cluster report: CD45/CD45R", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 628-634.
7. Terry, L.A., Brown, M.H., Beverley, P.C.L., "The monoclonal antibody, UCHL1, recognizes a 180,000 MW component of the human leucocyte-common antigen, CD45", 1988, Immunology, 64, 331-336.

PRODUCT AVAILABILITY

IOTest CD45RO-FITC Conjugated Antibody
PN IM1247U – 2 mL Liquid – 20 µL / test*.

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

Outside the USA, contact your local Beckman Coulter representative.

www.beckmancoulter.com

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

5 x 10⁵ cells in a standard immunofluorescence assay