



BD Multitest™

CD45RA/CD45RO/CD3/CD8

Catalog No. 340574

50 Tests

20 µL/test

RESEARCH APPLICATIONS

Research applications include studies of:

- Percentages and absolute counts of naive/memory T-suppressor cells^{1,2}

DESCRIPTION

Specificity

The CD45RA antibody recognizes a 220-kilodalton (kDa) molecular weight isoform of the leucocyte common antigen (LCA).³ The CD45RA antigen is a member of the CD45 antigen family that also includes the CD45, CD45RB, and CD45RO antigens.³

The CD45RO antibody, a member of the same CD45 family as CD45RA, recognizes a 180-kDa isoform of the LCA.⁴⁻⁸ The CD45 antigen is a protein tyrosine phosphatase.⁹

The CD3 antibody recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.¹⁰ This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kDa.¹¹ The antigen recognized by the CD3 antibody is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kDa).¹²

The CD8 antibody recognizes an antigen expressed on the 32-kDa α subunit of a disulfide-linked bimolecular complex.¹³ The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56^{lck}.¹⁴ The CD8 molecule interacts with class I major histocompatibility complex (MHC) molecules resulting in increased adhesion between the CD8⁺ T lymphocytes and the target cells.¹⁵⁻¹⁷ Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.¹⁵⁻¹⁷

Antigen distribution

The CD45RA antigen is present on approximately 50% of CD4⁺ T lymphocytes, approximately 75% of CD8⁺ T lymphocytes, and on essentially all B lymphocytes and natural killer (NK) lymphocytes.¹⁸ The suppressor inducer T-lymphocyte subset expresses the phenotype CD4⁺CD45RA⁺.¹⁸ The CD45RA antigen is expressed on naive T lymphocytes; antigen density decreases upon in vitro activation.¹⁹ A selective loss of the CD4⁺CD45RA⁺ subset during active multiple sclerosis has been demonstrated.^{20,21}

In peripheral blood, the CD45RO antigen is present on approximately 40% of resting peripheral blood T lymphocytes, including the CD4⁺ and CD8⁺ subpopulations, as well as on most thymocytes and activated T lymphocytes.^{4,5} It is also expressed on monocytes, macrophages, and granulocytes.^{4,5} The CD45RO antigen is present at low density early in the T-lymphocyte maturation cycle. Upon activation by phytohemagglutinin or alloantigen, naive T lymphocytes first acquire CD45RO and then lose CD45RA.^{5,6,22} When these activated T lymphocytes are rechallenged, the cells that exhibit a secondary response are primarily CD45RO⁺, leading to the concept that CD45RO⁺ cells are a primed population of memory T lymphocytes.^{5,6,22}

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

The CD3 antigen is expressed on 61% to 85% of normal peripheral blood lymphocytes,²³ 65% to 85% of thymocytes,²⁴ and on Purkinje cells in the cerebellum.²⁵

The CD8 antigen is expressed on the human suppressor/cytotoxic T-lymphocyte subset (CD3⁺CD8⁺),²⁶⁻³⁰ as well as on a subset of NK lymphocytes.³¹ The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes²³ and the majority of normal thymocytes.³²

Clones

The CD45RA antibody, clone L48, is derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells from BALB/c mice immunized with low-buoyant-density human lymphocytes.

The CD45RO antibody, clone UCHL-1, is derived from hybridization of P3/NS-1/1-Ag4-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with an interleukin-2 (IL-2)-dependent human T-cell line.⁴

The CD3 antibody, clone SK7,³³⁻³⁵ is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

The CD8 antibody, clone SK1, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

Composition

The CD45RA, CD3, and CD8 antibodies are each composed of mouse IgG₁ heavy chains and kappa light chains.

The CD45RO antibody is composed of mouse IgG_{2a} heavy chains and kappa light chains.

The BD Multitest reagent is supplied as a combination of CD45RA FITC, CD45RO PE, CD3 PerCP, and CD8 APC in 1.0 mL of phosphate buffered saline (PBS) containing bovine serum albumin (BSA), and 0.1% sodium azide.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

1. Pipet 20 µL of monoclonal antibody into a labeled tube.
2. Add 50 µL of whole blood.
3. Vortex gently to mix and incubate for 15 minutes in the dark at room temperature (20°C–25°C).
4. Add 1 mL of 1X BD FACS™ lysing solution (Cat. No. 349202) to the tube.

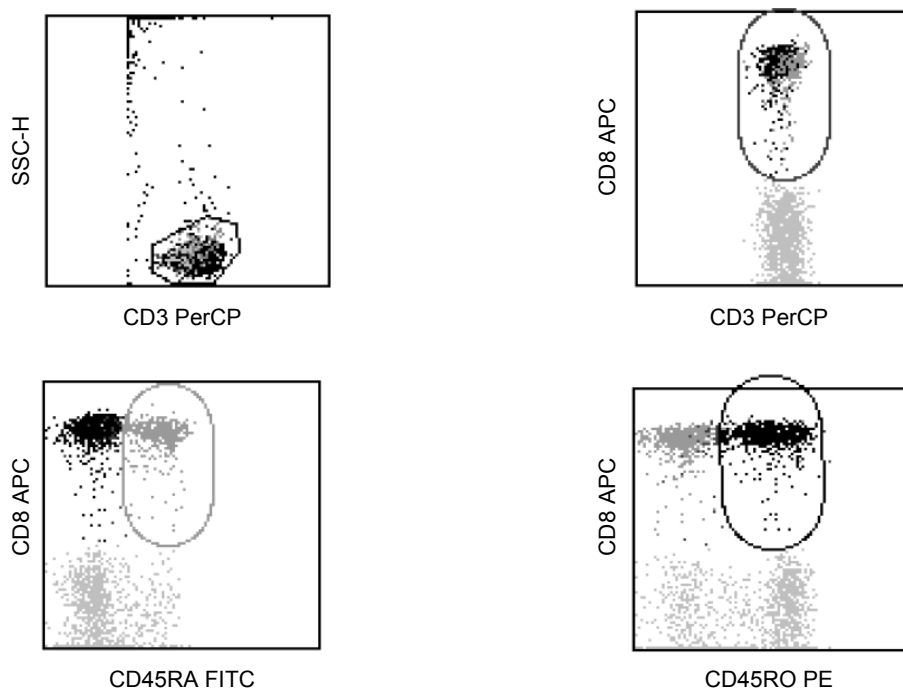
The volume of BD FACS lysing solution recommended has been optimized for use with this reagent.

5. Vortex gently and incubate for 15 to 30 minutes in the dark at room temperature. If samples are not analyzed immediately, mix thoroughly before analysis.

REPRESENTATIVE DATA

Performed on peripheral blood leucocytes using a BD FACSCalibur™ flow cytometer with a gate set on the CD3⁺ lymphocyte fraction. Laser excitation is at 488 nm and 635 nm.

Figure 1 Representative data analyzed with a BD FACS brand flow cytometer



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{36,37} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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