Monoclonal Antibodies Detecting Human Antigens

BD Simultest™
CD8/Anti-HLA-DR
Catalog No. 349528  50 Tests  20 µL/test

RESEARCH APPLICATIONS

Research applications* include studies of:
- Human immunodeficiency virus (HIV) disease¹⁻⁹
- Viral diseases such as those caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV)¹⁰⁻¹²
- Chronic inflammatory diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis¹¹,¹³
- Transplantation¹¹,¹³

DESCRIPTION

Specificity

The CD8 (Leu-2a) antigen is present on the human suppressor/cytotoxic T-lymphocyte subset¹⁴,¹⁵ as well as on a subset of natural killer (NK) lymphocytes.¹⁶ The CD8 antigenic determinant interacts with class I major histocompatibility complex (MHC) molecules resulting in increased adhesion between the CD8⁺ T lymphocytes and the target cells.¹⁷⁻¹⁹ Binding of the CD8 antigen to class I MHC molecules enhances the activation of resting T lymphocytes.¹⁷⁻¹⁹ CD8 recognizes an antigen expressed on the 32-kilodalton (kDa) α-subunit of a disulfide-linked bimolecular complex.²⁰ The cytoplasmic domain of the α-subunit of the CD8 antigen is associated with the protein tyrosine kinase p56lck.¹⁹,²¹

The HLA-DR antigen is a human class II MHC antigen. The antigen is a transmembrane glycoprotein composed of α- and β-subunits that have molecular weights of 36 and 27 kDa, respectively.²²,²³ The antibody reacts with a nonpolymorphic HLA-DR epitope.²²⁻²⁴

Antigen distribution

The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes²⁵ and 60% to 85% of normal thymocytes.¹⁴

The HLA-DR antigen is expressed on B lymphocytes, monocytes, macrophages, activated T lymphocytes, activated NK lymphocytes, and human progenitor cells.¹,¹⁰,₂⁶⁻²⁹ It is also present on thymic epithelium, B-lymphocyte–dependent areas of spleen and lymph node, and B-cell lymphomas.²⁷⁻³¹ The antigen is coexpressed with the CD1a antigen on Langerhans cells of the epidermis.³²

CD8⁺DR⁺ T lymphocytes are involved in the immune response to viral infections. Elevated levels of CD8⁺DR⁺ T lymphocytes are observed in HIV infection and during the acute stages of EBV infection and CMV infection. Increased numbers of CD8⁺DR⁺ lymphocytes are also exhibited in rheumatoid arthritis, SLE, graft-versus-host disease (GVHD), renal allograft rejection, and in the period immediately following bone marrow transplantation.²⁻¹³,³³,³⁴

* The published methods in the cited references have not been developed or tested by Becton Dickinson.

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In HIV infection, the coexpression of the CD8 and HLA-DR antigens, with a concomitant low percentage of CD4+ lymphocytes, is closely associated with disease progression.4,5 Beginning at seroconversion, and during the first stages of HIV infection, the number of CD8+ T lymphocytes increases while the number of CD4+ T lymphocytes decreases. When the onset of acquired immune deficiency syndrome (AIDS) occurs, there is a decline in the absolute CD3+, CD4+, and CD8+ lymphocyte levels along with a further decrease in the CD4+ T lymphocyte percentage, but the relative percentage of CD8+ T lymphocytes continues to rise until, in the late stages of AIDS, the majority of the remaining T lymphocytes are CD8+. The number of CD8+DR+ lymphocytes increases at the time of seroconversion, and remains elevated throughout the course of HIV infection. Peak levels occur at the AIDS-related complex (ARC) stage of the disease.1,2,4-8 CD8+DR+ T lymphocytes exhibit decreased activity of ecto-5'-nucleotidase (CD73 antigen), a maturation marker linked to T- and B-lymphocyte development.5 The majority of these lymphocytes express the VLA-2 antigen, a cell-surface marker associated with a chronic state of T-lymphocyte activation, but lack the IL-2 receptor (CD25 antigen), which is required for normal T-lymphocyte activation and clonal proliferation.9 CD8+DR+ lymphocytes exhibit a decreased clonogenic potential, and an inability to respond to mitogens such as phorbol 12-myristate 13-acetate (PMA), phytohemagglutinin (PHA), CD2, CD3, and CD28. Although these cells retain their HIV-specific cytolytic potential, their inability to proliferate results in a progressive decrease in their number, and the eventual loss of HIV-specific cytolytic activity.3,9

Clone

CD8, 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.15 Anti-HLA-DR, clone L243, is derived from hybridization of NS1/1-Ag4 mouse myeloma cells with spleen cells from BALB/c mice immunized with the human lymphoblastoid B-cell line RPMI 8866.22

Composition

The CD8 antigen is composed of mouse IgG1 heavy chains and kappa light chains.

The Anti-HLA-DR antigen is composed of mouse IgG2a heavy chains and kappa light chains.

The BD Simultest™ reagent is supplied as a combination of CD8 (Leu-2a) FITC and Anti-HLA-DR PE in 1.0 mL of phosphate buffered saline (PBS) containing gelatin 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.

Method for BD Simultest immunofluorescence

Peripheral Blood Mononuclear Cells (PBMCs): Add 20 µL of BD Simultest reagent to 10^6 PBMCs in 50 µL of medium containing 0.1% sodium azide. Mix thoroughly and incubate for 15 to 30 minutes in the dark at 2°C–8°C. Wash with 1X PBS with 0.1% sodium azide, add 0.5 mL of 1% paraformaldehyde, mix thoroughly, and analyze. If samples are not to be analyzed immediately, mix thoroughly just prior to analysis. See Becton Dickinson Procedure for Direct Immunofluorescence Staining of Cell Surfaces, Source Book Section 2.4.

NOTE

The occasional presence of cellular aggregates has been reported in PBMC preparations. For details, please refer to Becton Dickinson's technical update on The Escapee Phenomenon, Source Book Section 8.2.

Lysed Whole Blood (LWB): Add 20 µL of BD Simultest reagent to 100 µL of whole blood in a staining tube. Mix thoroughly and incubate 15 to 30 minutes in the dark at room temperature (20°C–25°C). Add 2 mL of 1X BD FACSTM Lysing Solution (Cat. No. 349202) at room temperature and vortex tube thoroughly. Incubate for no more than 10 to 12 minutes at room temperature in the dark. Wash cells with 1X PBS with
0.1% sodium azide, add 0.5 mL of 1% paraformaldehyde, mix thoroughly, and analyze. If samples are not to be analyzed immediately, mix thoroughly just prior to analysis. Refer to the BD FACS lysing solution package insert.

REPRESENTATIVE DATA

Performed on peripheral blood leucocytes with scatter gates set on the lymphocyte fraction. BD Simultest reagents contain two fluorochromes, FITC and PE. Both fluorochromes can be excited at 488 nm, yet will emit light at different wavelengths. However, spectral overlap of these populations does occur. To obtain optimal results with BD Simultest reagents, both green (FITC) and red-orange (PE) signals must be electronically compensated to correct for spectral overlap. Refer to the Becton Dickinson Procedure for Two-Color Fluorescence Analysis Using BD Simultest.

Figure 1 Two-parameter display of peripheral blood lymphocytes analyzed with a BD FACScan™ flow cytometer (Logarithmic Fluorescence Intensity)

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 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.

REFERENCES


