Monoclonal Antibodies
Detecting Human Antigens

BD Simultest™
CD8/CD38

Catalog No. 349527 50 Tests 20 µL/test

Research applications* include studies of:
• Human immunodeficiency virus (HIV) disease
• Diseases involving viral infection, such as those caused by Epstein-Barr virus (EBV) and Cytomegalovirus (CMV)1,2
• Transplantation

DESCRIPTION

Specificity
The CD8 (Leu-2a) antigen is present on the human suppressor/cytotoxic T-lymphocyte subset3,4 as well as on a subset of natural killer (NK) lymphocytes.5 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.6-8 Binding of the CD8 antigen to class I MHC molecules enhances the activation of resting T lymphocytes.6-8 CD8 recognizes an antigen expressed on the 32-kilodalton (kDa) α-subunit of a disulfide-linked bimolecular complex.9 The cytoplasmic domain of the α-subunit of the CD8 antigen is associated with the protein tyrosine kinase p56lck.8,10 CD38 (Leu-17) recognizes an antigen that is an integral membrane glycoprotein, with a molecular weight of 45 kDa, with a protein core of 35 kDa.11

Antigen distribution
The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes12 and 60% to 85% of normal thymocytes.3 The CD38 antigen is expressed on essentially all pre-B lymphocytes, plasma cells, and thymocytes. It is also present on activated-T lymphocytes, NK lymphocytes, monocytes, myeloblasts, and erythroblasts.1,2,11,13,14,15-19 The antigen is expressed during the early stages of T- and B-lymphocyte differentiation, is lost during the intermediate stages of maturation, and then reappears during the final stages of maturation.2,11,18,20 The antigen is also expressed in some cases of T- and B-acute lymphoblastic leukemia (ALL), Burkitt’s lymphoma, multiple myeloma, and acute myeloid leukemia (AML).17,21 CD8+CD38+ T lymphocytes are involved in the immune response to viral infection. Elevated levels of CD8+CD38+ lymphocytes are observed in HIV infection, and during the acute stages of EBV and CMV infection.1,2 Increased numbers of CD8+CD38+ T lymphocytes are also present in the period immediately following bone marrow transplantation.22

In HIV infection, the coexpression of the CD8 and CD38 antigens, with a concomitant low percentage of CD4+ lymphocytes, is closely associated with disease

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

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
progression. 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+. Elevated levels of CD8+CD38+ T lymphocytes become apparent at the time of seroconversion and continue to rise throughout the course of the disease, indicating persistent stimulation of the immune system by the virus. CD8+CD38+T lymphocytes exhibit decreased activity of ecto-5′-nucleotidase (CD73 antigen), a maturation marker linked to T- and B-lymphocyte development, and appear to be activated and/or immature suppressor/cytotoxic-T lymphocytes.

Clones

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.

The CD38 antibody, clone HB-7, is derived from hybridization of P3-X63-Ag8.653 mouse myeloma cells with spleen cells from BALB/c mice immunized with the BJAB cell line.

Composition

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

The CD38 antibody is composed of mouse IgG1 heavy chains and kappa light chains.

The BD Simultest™ reagent is supplied as a combination of CD8 (Leu-2a) FITC and CD38 (Leu-17) 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. Refer to 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 FACS™ 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

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\textsuperscript{23,24} 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.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD’S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

REFERENCES

   AIDS. 1990;4:479-497.
2. Salazar-Gonzalez JF, Moody DJ, Giorgi JV, Martinez-Maza O, Mitsuyasu RT, Fahey JL. Reduced ecto-5’
   -nucleotidase activity and enhanced OKT10 and HLA-DR expression on CD8 (T suppressor/cytotoxic)
   lymphocytes in the acquired immune deficiency syndrome: Evidence of CD8 cell immaturity. 
   conservation of surface molecules that distinguish T-lymphocyte helper/inducer and T
4. Evans RL, Wall DW, Platsoucas CD, et al. Thymus-dependent membrane antigens in man: Inhibition of
   cell-mediated lympholysis by monoclonal antibodies to the TH2 antigen. Proc Natl Sci Acad USA.
   defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. J Immunol. 1983;131:1789-
   1796.


