Monoclonal Antibodies Detecting Human Antigens

BD Simultest™
CD4/Leu-8

Catalog No. 349513 50 Tests 20 µL/test

RESEARCH APPLICATIONS

Research applications include studies of:

- Analysis of immunoregulatory T-lymphocyte subsets involved in helper/inducer functions
- Enumeration and monitoring of T-lymphocyte subset levels in peripheral blood of subjects with immunologic abnormalities

DESCRIPTION

Specificity

The CD4 (Leu-3a) antigen, with a molecular weight of 55 kilodaltons (kDa), is present on T-helper/inducer lymphocytes and monocytes. Leu-8 is a human leucocyte antigen, with a molecular weight of 80 kDa.

Antigen distribution

The CD4 (Leu-3a) antigen is present on the helper/inducer T-lymphocyte subset (CD3+CD4+) that comprises 28% to 58% of normal peripheral blood lymphocytes. It is also present on 80% to 95% of normal thymocytes. The CD4 (Leu-3a) antigen is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes and macrophages (CD3-CD4+). The CD4 antigen is the receptor for the human immunodeficiency virus (HIV). CD4 (Leu-3a) inhibits HIV binding to CD4+ cells. Patients with acquired immunodeficiency syndrome (AIDS) were found to exhibit T-cell lymphopenia along with a continuous loss of CD4+ lymphocytes and a relative increase in the CD8 (Leu-2a+) lymphocyte subset. AIDS patients may also display an expansion of a CD3+CD4+CD8- T-lymphocyte subset.

The Leu-8 antigen is present on 68% ± 7% of normal human peripheral blood lymphocytes, 70% to 80% of E-rosetting cells, 10% of thymocytes, and most B lymphocytes. Leu-8 also reacts with monocytes, granulocytes, and a subset of natural killer (NK) lymphocytes.

Regulatory subpopulations of T lymphocytes are defined by the CD4 (Leu-3a) and Leu-8 antigens. Approximately 75% of CD4+ T lymphocytes also bear the Leu-8 antigen. CD4+Leu-8+ lymphocytes mediate the majority of helper functions involved in B-lymphocyte differentiation into plaque-forming cells, whereas the suppressor inducer functional subpopulation is included in the CD4+Leu-8+ compartment.

Clone

The CD4 (Leu-3a) antigen, clone SK3, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood lymphocytes.

* 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.
The Leu-8 antigen, clone SK11, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/C mice immunized with peripheral blood T lymphocytes.

Composition

The CD4 (Leu-3a) antigen is composed of mouse IgG1 heavy chains and kappa light chains.

The Leu-8 antigen is composed of mouse IgG2a heavy chains and kappa light chains.

The BD Simultest™ reagent is supplied as a combination of CD4 (Leu-3a) FITC and Leu-8 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.

Peripheral Blood Mononuclear Cells (PBMC): Add 20 µL of BD Simultest reagent to 10^6 PBMC 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 twice with 1X PBS with 0.1% sodium azide, add 0.5 mL of 1% paraformaldehyde, mix thoroughly, and analyze. If samples will not 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 the Becton Dickinson 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 at room temperature and vortex tube thoroughly. Incubate for no more than 10 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 will not be analyzed immediately, mix thoroughly just prior to analysis. Refer to the BD FACSTM Lysing Solution package insert.

NOTE  Values for leucocyte subsets obtained with PBMCs may differ from values obtained with lysed whole blood.

CAUTION  Store blood at room temperature prior to staining. Samples should be fixed immediately after staining. Analyze within 24 hours to minimize loss of the Leu-8 antigen.

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 488nm, 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 Reagents, Source Book Section 2.13.
Figure 1  Two-parameter display of peripheral blood lymphocytes (LWB) 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\(^{18,19}\) 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


