Monoclonal Antibodies
Detecting Human Antigens

BD Simultest™
Anti-Kappa/Anti-Lambda

Catalog No. 349516  50 Tests  20 μL/test

RESEARCH APPLICATIONS

Research applications* include studies of:

• Simultaneous enumeration of B lymphocytes bearing kappa light chains and B lymphocytes bearing lambda light chains in peripheral blood

• Clonality of B-cell leukemias or lymphomas in peripheral blood or tissue

• Analysis of Fc receptor–positive, cytophilic Ig-binding cells (kappa and lambda double-positive cells)^2^

DESCRIPTION

Specifity

Anti-Kappa is specific for kappa light chains of human immunoglobulins.

Anti-Lambda is specific for lambda light chains of human immunoglobulins.

Antigen distribution

Immunoglobulins bearing kappa light chains are present on approximately 50% of normal human B lymphocytes and on Igκ+ leukemic cells. Lambda light chains are present on approximately 50% of normal human B lymphocytes and on Igλ+ leukemic cells.1-9

Clones

Anti-Kappa: TB28-2 is derived from hybridization of P3-X63-Ag8.653 mouse myeloma cells with cells from CB6 (BC57b X BALB/c) mice immunized with human IgG, κ myeloma protein.

Anti-Lambda: 1-155-2 is derived from hybridization of P3-X63-Ag8.653 mouse myeloma cells with cells from BALB C/J mice immunized with human IgA1, λ myeloma protein.

Composition

Anti-Kappa is composed of mouse IgG1 heavy chains and kappa light chains.

Anti-Lambda is composed of mouse IgG1 heavy chains and kappa light chains.

The BD Simultest™ reagent is supplied as a combination of Anti-Kappa FITC and Anti-Lambda PE in 1.0 mL of phosphate buffered saline 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 two-color 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

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

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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. To eliminate staining of lymphocytes that bind serum cytophilic Ig by Fc binding, follow Becton Dickinson Procedure for Blockage of Serum Cytophilic Ig Binding, Source Book Section 2.15.

**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.

**Lyzed 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 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 FACS 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**

Flow cytometric analysis was 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 Reagents, Source Book Section 2.13.

**NOTE**  BD Simultest Anti-Kappa/Anti-Lambda is not for use in the laboratory diagnosis of “clonal excess.” The evaluation of clonally-expanded B lymphocytes is performed by direct comparison of fluorescence distribution curves obtained from cells stained separately with Anti-Kappa and Anti-Lambda and with the same fluorochrome.

Also, since Anti-Kappa and Anti-Lambda will react with human Ig bound to cells with Fc receptors (ie, platelets, monocytes, natural killer (NK) cells, and B lymphocytes) some cells will be double-stained. The extent of this double staining will vary from donor to donor and can be influenced by such factors as monocytes present in the lymphocyte gate, relative percentage of NK lymphocytes, and presence of monoclonal immunoglobulins in donor plasma. To reduce this cytophilic staining, refer to Becton Dickinson Procedure for Blockage of Serum Cytophilic Ig Binding, Source Book Section 2.15. See Figure 1.

In addition, those B lymphocytes expressing surface Ig and Fc receptors will have distinctive Anti-Kappa and Anti-Lambda staining but may demonstrate a slight shift toward the double-stained population.
Figure 1 Two-parameter display of peripheral blood lymphocytes (LWB) 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


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