Monoclonal Antibodies
Detecting Human Antigens

BD Multitest™

CD8/CD38/CD3/Anti–HLA-DR

Catalog No. 340572
50 Tests
20 µL/test

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

Research applications include studies of percentages and absolute counts of:

- Activated T lymphocytes¹⁻⁴
- Activated T-suppressor cells in AIDS⁵⁻⁹,¹⁰⁻²⁰
- Diseases involving viral infection, such as those caused by EBV and CMV⁶,⁸,²¹⁻²³
- Transplantation²²,²⁴
- Chronic inflammatory diseases such as SLE and rheumatoid arthritis²⁵,²⁶

DESCRIPTION

Specificity

The CD8 antibody recognizes an antigen expressed on the 32-kilodalton (kDa) α subunit of a disulfide-linked bimolecular complex.²⁷ The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56ck.²⁸ 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.²⁹⁻³¹ Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.²⁹⁻³¹

The CD38 antibody recognizes an integral membrane glycoprotein, with a molecular weight of 45 kilodaltons (kDa) and a protein core of 35 kDa.³²

The CD3 antibody recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.³³ This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kDa.³⁴ The antigen recognized by the CD3 antibody is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kDa).³⁵

The Anti–HLA-DR antibody recognizes a human class II MHC antigen.³⁶,³⁷ The antigen is a transmembrane glycoprotein composed of α and β subunits that have molecular weights of 36 and 27 kDa, respectively.³⁶,³⁷ Anti–HLA-DR reacts with a nonpolymorphic HLA-DR epitope³⁶⁻³⁸ and does not cross-react with HLA-DQ or HLA-DP molecules.³⁸

Antigen distribution

The CD8 antigen is expressed on the human suppressor/cytotoxic T-lymphocyte subset (CD3⁺CD8⁺),³⁹⁻⁴³ as well as on a subset of natural killer (NK) lymphocytes.⁴⁴ The CD8 antigen is expressed on 19% to 48% of normal peripheral blood lymphocytes⁴⁵ and the majority of normal thymocytes.⁴⁶

The CD38 antigen is expressed on essentially all pre-B lymphocytes, plasma cells, and thymocytes.³² It is also present on activated T lymphocytes, NK lymphocytes, myeloblasts, and erythroblasts.⁵⁻⁷,³²,⁴⁸⁻⁵⁰ 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.⁸,³²,⁵⁰,⁵¹ The
CD38 antigen is expressed on 90% of CD34+ cells and is not expressed on pluripotent stem cells. Coexpression of CD38 antigen on CD34+ cells indicates lineage commitment of those cells.\textsuperscript{8,48} It is also expressed in T- and B-acute lymphoblastic leukemia, Burkitt's lymphoma, multiple myeloma, and acute myeloid leukemia.\textsuperscript{52,53}

The CD3 antigen is expressed on 61% to 85% of normal peripheral blood lymphocytes,\textsuperscript{45} 65% to 85% of thymocytes,\textsuperscript{54} and on Purkinje cells in the cerebellum.\textsuperscript{21}

The HLA-DR antibody is expressed on B lymphocytes, monocytes, macrophages, activated T lymphocytes, activated NK lymphocytes, and human progenitor cells.\textsuperscript{9,55-59} It is also present on thymic epithelium, B-lymphocyte–dependent areas of spleen and lymph node, and B-cell lymphomas.\textsuperscript{58-62} The antigen is coexpressed with the CD1a antigen on Langerhans cells of the epidermis.\textsuperscript{46}

### 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 HB7, 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.\textsuperscript{50}

The CD3 antibody, clone SK7,\textsuperscript{63-65} is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

The Anti–HLA-DR antibody, clone L243, is derived from the hybridization of NS-1/1-Ag4 mouse myeloma cells with spleen cells from BALB/c mice immunized with the human lymphoblastoid B-cell line RPMI 8866.\textsuperscript{36}

### Composition

The CD8, CD38, and CD3 antibodies are each composed of mouse IgG\textsubscript{1} heavy chains and kappa light chains.

The Anti–HLA-DR antibody is composed of mouse IgG\textsubscript{2a} heavy chains and kappa light chains.

The BD Multitest reagent is supplied as a combination of CD8 FITC, CD38 PE, CD3 PerCP, and Anti–HLA-DR 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/no-wash protocol for direct immunofluorescence.

**Lyse/No-Wash method for direct immunofluorescence**

1. Pipet 20 µL of monoclonal antibody into a labeled tube.
   
   For absolute counts, use BD Trucount Tubes. Refer to the BD Trucount Tubes package insert for more detailed information.

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 FACSTM 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

Flow cytometric analysis was performed on peripheral blood leukocytes using a BD FACS\textsuperscript{Calibur\textsuperscript{TM}} flow cytometer with a gate set on the CD3\textsuperscript{+} lymphocyte fraction. Laser excitation was at 488 nm and 635 nm.
**Figure 1** Representative data analyzed with a BD FACSCalibur 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 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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**REFERENCES**


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