Monoclonal Antibodies Detecting Human Antigens

BD FastImmune™
CD8/CD69/CD3
Catalog No. 340367  50 Tests  20 µL/test

RESEARCH APPLICATIONS

Research applications include studies of:

- Activated T lymphocytes and T-lymphocyte subsets (helper/suppressor) in peripheral blood
- T-lymphocyte activation mechanisms

DESCRIPTION

Specificity

The CD8 (Leu-2a) antibody recognizes the 32-kilodalton (kDa) α subunit of a disulfide-linked bimolecular complex of the CD8 antigen. The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56lck. The CD8 molecule interacts with class I major histocompatibility complex (MHC) molecules resulting in increased adhesion. Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.

The CD69 antibody recognizes a very early human lymphocyte activation antigen. The CD69 antigen is a surface homodimer formed by the association of 28-kDa and 32-kDa chains that are held together by disulfide bridges.

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

Antigen distribution

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

The CD69 antigen is present on activated T, B, and NK lymphocytes and platelets. CD69 is not expressed by resting peripheral blood lymphocytes (PBLs). Upon activation, CD69 antigen expression increases on lymphocytes; peak expression generally occurs within 18 hours, preceding the appearance of HLA-DR, interleukin-2 (IL-2) receptor (CD25 antigen), and transferrin receptor (CD71 antigen). CD69 and phorbol ester are comitogenic for T lymphocytes. In thymus, the CD69 antigen is constitutively expressed on the bright CD3+ subset.

The CD3 antigen is expressed on 61% to 85% of normal peripheral blood lymphocytes, 65% to 85% of thymocytes, and on Purkinje cells in the cerebellum.

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.

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The CD69 antibody, clone L78, is derived from hybridization of Sp2/0-Ag14 mouse myeloma cells with lymph node cells from BALB/c mice immunized with a CD8+ alloantigen-directed cytotoxic T-lymphocyte (CTL) cell line.28

The CD3 antibody, clone SK7,13,29-31 is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

Composition
The CD8, CD69, and CD3 antibodies are each composed of mouse IgG1 heavy chains and kappa light chains.

The BD FastImmune reagent is supplied as a combination of CD8 FITC, CD69 PE, and CD3 PerCP 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/wash protocol for direct immunofluorescence.

Method for direct immunofluorescence
After the activation process, pipet 20 µL of BD FastImmune CD8/CD69/CD3 reagent into a labeled tube. Add 50 µL of whole blood. Vortex gently to mix and incubate for 15 minutes in the dark at room temperature. Add 450 µL of 1X BD FACSTM lysing solution (Cat. No. 349202) to the tube. Vortex gently and incubate for 15 to 30 minutes in the dark at room temperature.

Contact your local BD representative for our procedure, Flow Cytometric Procedure for Assessing Lymphocyte Activation, for the complete activation protocol. This procedure follows the basic format described previously.32

REPRESENTATIVE DATA
Flow cytometric analysis was performed on lysed whole blood with a gate (R1) set on the CD3+ lymphocyte fraction. Laser excitation was at 488 nm.

Figure 1 Four-hour CD2/CD2R–activated CD3+ lysed whole blood 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 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


