Monoclonal Antibodies Detecting Human Antigens

BD FastImmune™
CD69/CD3
Catalog No. 340368  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

NOTE  BD FastImmune CD69/CD3 is an open reagent for the BD FastImmune System that allows customized subset analysis. The functions of specific T-lymphocyte subpopulations can be studied by adding FITC-labeled T-lymphocyte subset antibodies to this two-color reagent.

Specificity

The CD69 antibody recognizes a very early human lymphocyte activation antigen. The CD69 antigen is a surface homodimer formed by the association of 28-kilodalton (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 CD69 antigen is present on activated T, B, and natural killer (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 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.

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

Composition

The 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 CD69 PE and CD3 PerCP in 1.0 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and 0.1% sodium azide.

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

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

After the activation process, pipet 20 µL of BD FastImmune CD69/CD3 reagent and the appropriate amount of FITC-labeled antibody, if desired, 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 FACS™ lysing solution (Cat. No. 349202) to the tube. Vortex gently and incubate for 15 to 30 minutes in the dark at room temperature.

Visit our website (bdbiosciences.com) or contact your local BD representative for the BD procedure Flow Cytometric Procedure for Assessing Lymphocyte Activation for the complete activation protocol. This procedure follows the basic format described previously.20

REPRESENTATIVE DATA

Performed on lysed whole blood with a gate (R1) set on the CD3+ lymphocyte fraction. Laser excitation is 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 infection21,22 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


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