RESEARCH APPLICATIONS

BD FastImmune™ Anti-Hu–IFN-γ/CD69/CD4/CD3 is designed for the detection of intracellular cytokines and expression of the activation marker CD69 in antigen-activated CD4+ T lymphocytes in whole blood. Applications include studies of T-cell responses to antigens such as cytomegalovirus (CMV),1-5 human immunodeficiency virus (HIV),6,7 herpes viruses,1 and tumor antigens.8

DESCRIPTION

Specificity

Anti-Human Interferon-γ (Anti-Hu–IFN-γ) recognizes a 20- to 25-kilodalton (kDa) glycoprotein.9 The CD69 antibody recognizes a very early human activation antigen. The CD69 antigen is a surface homodimer formed by the association of 28- and 32-kDa chains that are held together by disulfide bridges.10 The CD411,12 antibody recognizes an antigen with a molecular weight of 55 kDa13 that is present on T-helper/inducer lymphocytes and monocytes.14,15 The CD3 antibody reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.16 This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kDa.17 The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γδ TCR (70 to 90 kDa).18

Antigen distribution

IFN-γ is produced, upon activation, by most CD8+ T lymphocytes, by the Th1 and Th0 subsets of CD4+ T lymphocytes, and by natural killer (NK) lymphocytes.9,19-21 IFN-γ is a multifunctional immunomodulator with anti-tumor and anti-viral activity.22,23 IFN-γ is a pleiotropic cytokine instrumental in the regulation of immune and inflammatory processes,24-26 and it has a role in the differentiation and function of monocytes.26 The CD69 antigen is present on activated T, B, and NK lymphocytes10 and platelets.27 In normal peripheral blood, a variable percentage of lymphocytes express the CD69 antigen.8 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).28-30 CD69 and phorbol ester are comitogenic for T lymphocytes.30 In thymus, the CD69 antigen is constitutively expressed on the bright CD3+ subset.31

The CD4 antigen is present on the helper/inducer T-lymphocyte subset, such as CD3+CD4+, that comprises 28% to 58%32 of normal peripheral blood lymphocytes.13,15 It is also present on 80% to 95% of normal thymocytes.13,15 The CD4 antigen is present in low density on the cell surface of monocytes and in the

* Due to the variable density of the antigen, the number of positive events can vary depending upon the brightness of the fluorochrome and the sensitivity of the instrument.

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cytoplasm of monocytes and macrophages (CD3\(^{-}\)CD4\(^{+}\)).\(^33\) The CD4 antigen is the receptor for the HIV.\(^34\) Some CD4 antibodies, including CD4, inhibit HIV binding to CD4\(^{+}\) cells.\(^35\) Subjects infected with HIV were found to exhibit a continuous loss of CD4\(^{+}\) lymphocytes and a relative increase in the CD8 (Leu-2a\(^{+}\)) lymphocyte subset.\(^36-38\)

The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes.\(^32\)

**Clones**

Anti-Hu–IFN-\(\gamma\), clone 25723.11, is derived from the hybridization of P3X-63-Ag8.653 mouse myeloma cells with lymph node cells from BALB/c mice immunized with recombinant human IFN-\(\gamma\).

CD69, 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.\(^39\)

CD4, clone SK3, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

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

**Composition**

Anti-Hu–IFN-\(\gamma\) is composed of mouse IgG\(_{2b}\) heavy chains and kappa light chains.

CD69, CD4, and CD3 are each composed of mouse IgG\(_{1}\) heavy chains and kappa light chains.

The BD FastImmune™ Anti-Hu–IFN-\(\gamma\)/CD69/CD4/CD3 reagent is supplied as a combination of IFN-\(\gamma\) FITC, CD69 PE, CD4 PerCP-Cy\(_{5.5}\), and CD3 APC in 1.0 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA), beta-lactoglobulin, and 0.1% sodium azide.

**PROCEDURE**

For complete activation and staining protocol and the appropriate application note, visit our website (bdbiosciences.com) or contact your local BD representative.

**Method for Intracellular Cytokine Detection**

**Abbreviated Intracellular Staining Procedure** – Add 1 mL of 1X BD FACS™ lysing solution (Cat. No. 349202) to 100 \(\mu\)L of activated heparinized whole blood. Incubate for 10 minutes at room temperature. Centrifuge at 500 \(x\)\(g\) for 5 minutes; decant the supernatant. Add 0.5 mL of 1X BD FACS™ Permeabilizing Solution 2 (Cat. No. 347692). Vortex and incubate for 10 minutes at room temperature. Wash by adding PBS containing 0.5% BSA and 0.1% sodium azide, and centrifuge for 5 minutes. Add 20 \(\mu\)L of BD FastImmune Anti-Hu–IFN-\(\gamma\) FITC/CD69 PE/CD4 PerCP-Cy\(_{5.5}\)/CD3 APC. Vortex and incubate for 30 minutes at room temperature in the dark. Repeat wash step; resuspend cells in 1% paraformaldehyde in PBS.

**REPRESENTATIVE DATA**

Performed on SEB-activated whole blood. Laser excitation is at 488 nm and 635 nm.

**Figure 1** Representative data analyzed with a BD FACS™ 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\(^{40,41}\) 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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