Monoclonal Antibodies Detecting Human Antigens

BD FastImmune™ Anti-Human TNF-α (6401.1111)

Form	Catalog number
FITC	340511
PE	340512
APC	340534

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include studies of:

- T- and B-lymphocyte interactions and immunoglobulin production[1]
- Pathogenesis of chronic inflammatory diseases[2,3]
- T- and B-lymphocyte activation, proliferation, and differentiation[4-6]
- Cytokines and their inhibitors[7,8]
- Acquired immune deficiency syndrome (AIDS) pathogenesis[9,10]
- Sepsis[11]

DESCRIPTION

Specificity

The BD FastImmune™ Anti-Human Tumor Necrosis Factor-α (Anti-Hu–TNF-α) antibody recognizes a 26-kilodalton (kDa) transmembrane protein.[1]

Antigen distribution

The BD FastImmune Anti-Hu–TNF-α antibody, formerly known as cachectin, is a pleiotropic cytokine that is a major mediator of inflammatory, immunological, and pathophysiological reactions.[2,12] Two distinct species of the molecule exist, a 26-kDa membrane expressed form (mTNF) and a 17-kDa soluble form (sTNF). [13-16] The sTNF is derived from the proteolytic cleavage of the mTNF.[13] Both forms are bioactive, with the active form of the sTNF being composed of a trimer of identical 17-kDa subunits.[15,16] The 17-kDa form, bound to its receptor, can also be detected on the membrane of activated cells.[15] There are two distinct membrane receptors for TNF, both of which help to mediate the cellular effects of TNF-α; one receptor has a molecular weight of 55 to 60 kDa (p55 or TNF-R1) and the other receptor has a molecular weight of 70 to 80 kDa (p75 or TNF-R2).[4,17-20]

The BD FastImmune Anti-Hu–TNF-α antibody is primarily produced by activated lymphocytes and macrophages.[21-23] In T lymphocytes, TNF-α can be induced through different molecular pathways, including the AIM/CD69 activation pathway and the CD3 and CD28 pathway.[5,24] Lipopolysaccharide (LPS) and other endotoxins are potent triggers of TNF-α from macrophages/monocytes.[25,26]

The BD FastImmune Anti-Hu–TNF-α antibody is a potent proinflammatory polypeptide that exhibits multiple biological activities.[2,12,27,28] It contributes to the defense against infectious agents and the control of tumor growth.[2,13,15] TNF-α displays antiviral activity, has a role in enhancing eosinophil function, and is involved in the activation of antileishmanial defense.[29-33] Conversely, TNF-α is implicated as a major mediator of septic shock, rheumatoid arthritis, inflammatory tissue destruction, and of cachexia associated with chronic disease states.[2,12,25,28] It has been postulated that different forms of the TNF molecule induce different physiologic responses, with

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the cell-bound version (26 kDa) being advantageous to the host and the secreted version (17 kDa) being deleterious.[13,14,18,34]

Clone

The BD FastImmune Anti-Hu–TNF-α antibody, clone 6401.1111,[1] is derived from a fusion of Sp2/0 myeloma cells with splenocytes from BALB/c mice immunized with recombinant human TNF-α.

Composition

The BD FastImmune Anti-Hu–TNF-α antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	3.2	1.0	3.2	Gelatin	0.1% Sodium azide
PE	50	20	3.2	1.0	3.2	Gelatin	0.1% Sodium azide
APC	100	5	6.25	0.5	12.5	Gelatin	0.1% Sodium azide

a. Volume required to stain 10^6 cells.

PROCEDURE

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

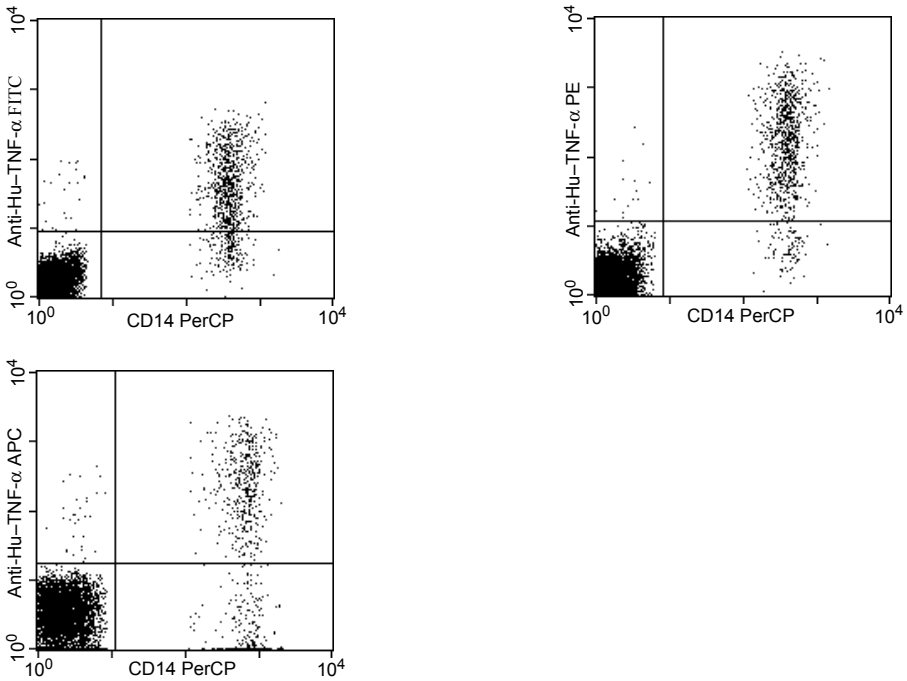
Method for Intracellular Cytokine Detection

Abbreviated Intracellular Staining Procedure: After surface staining activated whole blood with fluorescent-conjugated monoclonal antibodies, lyse the red blood cells by adding 2 mL of 1X BD FACS™ lysing solution (Cat. No. 349202). Vortex gently and incubate 5 to 10 minutes at room temperature. Centrifuge at 500 x *g* for 5 minutes; remove the supernatant. Add 500 µL of 1X BD FACS™ Permeabilizing Solution 2 (Cat. No. 347692). Vortex and incubate for 10 minutes at room temperature in the dark. Wash by adding PBS containing 0.5% bovine serum albumin (BSA) and 0.1% sodium azide (NaN_3), and centrifuge for 5 minutes. Add fluorescent-conjugated intracellular antibodies. Vortex and incubate for 30 minutes at room temperature in the dark. Repeat wash step. Resuspend cells in 1% paraformaldehyde in PBS.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on activated lysed whole blood with a gate set on mononuclear cells. Laser excitation was at 488 nm and 635 nm. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.

Figure 1 Four-hour LPS–activated lysed whole blood 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[35,36] 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.

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PATENTS AND TRADEMARKS