Monoclonal Antibodies Detecting Human Antigens

B-D®

FastImmune™ Anti-BrdU* with DNase

FITC | Catalog No. 340649 | 50 Tests

DESCRIPTION

Specificity

Bromodeoxyuridine (BrdU) is a uridine derivative that can be incorporated specifically into DNA in place of thymidine. Anti-BrdU identifies BrdU (but not thymidine) in single-stranded DNA, free BrdU, or BrdU coupled to a protein carrier. The antibody also reacts with iodouridine.[1,2] DNase-I cleaves each strand of DNA at random and permits the binding of Anti-BrdU antibody to its antigen (BrdU).

Clone

Anti-BrdU, clone B44, is derived from hybridization of mouse Sp2/0-Ag14 myeloma cells with spleen cells from BALB/c mice immunized with iodouridine-conjugated ovalbumin.[1]

Ig Chain Composition

Anti-BrdU is composed of mouse IgG_1 heavy chains and kappa light chains.

NOTE: Cells can be pulse-labeled with BrdU, and those cells that are synthesizing DNA (in S-phase of the cell cycle) will incorporate BrdU into the DNA. Anti-BrdU can then be used to identify cells that undergo DNA synthesis during exposure to BrdU. The proportion of cells in S-phase of the cell cycle can be determined either by fluorescence microscopy or by flow cytometric analysis.[1,3]

RESEARCH APPLICATIONS

Studies of:

- lymphocyte subsets based on proliferation at single cell level[3†]
- mitogen induced T-cell activation[4]
- antigen specific T-cell responses[5,6]
- immunopathogenesis of infectious diseases[7]

DIRECT IMMUNOFLUORESCENCE

Product/Amount for Staining

FastImmune Anti-BrdU FITC with DNase
Cat. No. 340649
20 µL/test

Method for Direct Immunofluorescence

Refer to the Becton Dickinson application note *Simultaneous Detection of Proliferation and Cytokine Expression in PBMCs* for the complete protocol for activation, staining, and analysis.

NOTE: To secure a fully-active enzyme, do not vigorously stir the reagent at any point in the procedure.

Abbreviated Activation and Staining Procedure: Activate peripheral blood mononuclear cells (PBMCs) in 15-mL polypropylene tubes by incubating in 5% $C0_2$ at a 5° slant from horizontal in the appropriate stimuli for approximately 36 to 40 hours at 37°C. Add BrdU and incubate further for 5 to 6 hours. Harvest the cells by adding EDTA and cold phosphate-buffered saline (PBS). Resuspend the pellet in 3 mL FACS™ Lysing Solution‡ (Cat. No. 349202). Vortex gently and incubate for 10 minutes at room temperature (20° to 25°C). Centrifuge at 500 x *g* for 10 minutes. Resuspend the pellet in 1 mL of PBS with 1%BSA and 0.1% azide and aliquot 100 µL/test. Add 500 µL of FACS Permeabilizing Solution (Cat. No. 340457). Vortex and incubate for 10 minutes at room temperature. Wash by adding PBS with 1% BSA and 0.1% sodium azide.

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* US Patent No. 4,529,700.
† Application is compatible with simultaneous detection of surface markers and intracellular markers.
‡ US Patent Nos. 4,654,312; 4,902,613; and 5,098,849.

For research use only. Not for use in diagnostic or therapeutic procedures.

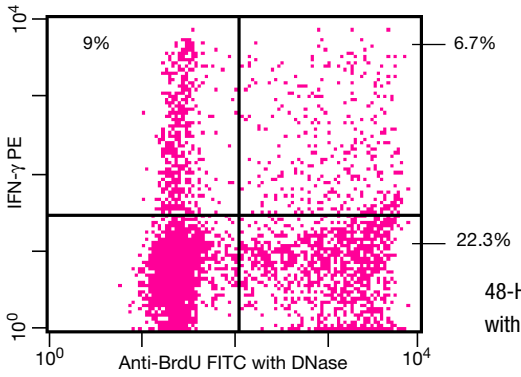
BECTON DICKINSON

Becton Dickinson Immunocytometry Systems
2350 Qume Drive
San Jose, CA 95131-1807
Ordering information (800) 223-8226; Customer Support Center (800) 448-BDIS

Add 20 µL of fluorescence-conjugated Anti-BrdU with DNase (nuclear staining) and surface staining antibodies at recommended concentrations. Vortex and incubate for 30 minutes in the dark at room temperature. Repeat wash step and resuspend the cells in 200 µL 1% paraformaldehyde in PBS. Mix thoroughly and analyze cells on a flow cytometer, gating on live cells (scatter) and triggering on FL3, for example, CD4 PerCP.

Flow Cytometric Method

Performed on activated normal PBMCs gated on the $CD4^+$ T-lymphocyte population. Laser excitation is at 488 nm.



48-Hour SEB–Activated Normal PBMCs Analyzed with a FACS® Brand Flow Cytometer

HANDLING AND STORAGE

The FITC conjugate is supplied as 10.0 µg in 1.0 mL (10 µg/mL) of balanced salt solution containing DNase, gelatin, and 0.1% sodium azide. Vials should be stored at 2° to 8°C. Conjugated forms should **not** be frozen and should be protected from prolonged exposure to light. Each reagent is stable for the period shown on the bottle label when stored as directed.

WARRANTY

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. Becton Dickinson's sole liability is limited to either replacement of the products or refund of the purchase price. Becton Dickinson is not liable for property damage, personal injury, or economic loss caused by the product.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data are included in this data sheet.

WARNING

Reagents contain sodium azide. Sodium azide is harmful if swallowed. Keep out of reach of children. Keep away from food, drink, and animal feedingstuff. Wear suitable protective clothing. If swallowed, seek medical advice immediately and show this container or label. Contact with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions may develop.

REFERENCES

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3. Mehta BA, Maino VC. Simultaneous detection of DNA synthesis and cytokine production in staphylococcal enterotoxin B activated $CD4^+$ T lymphocytes by flow cytometry. *J Immunol Methods.* 1997;208:49-59.
4. ElGhazali GEB, Paulie S, Andersson G, et al. Number of interleukin-4– and interferon-γ–secreting human T cells reactive with tetanus toxoid and the mycobacterial antigen PPD or phytohemagglutinin: distinct response profiles depending on the type of antigen used for activation. *Eur J Immunol.* 1993;23:2740-2745.
5. Waldrop S, Pitcher C, Peterson D, Maino V, Picker L. Determination of antigen-specific memory/effector $CD4^+$ T cell frequencies by flow cytometry. *J Clin Invest.* 1997;99:1739-1750.
6. Murlidhar G, Koch S, Broome HE, Swain SL. TCR triggering of anergic CD4 T cells in murine AIDS induces apoptosis rather than cytokine synthesis and proliferation. *J Immunol.* 1996;157:625-635.
7. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1–specific $CD4^+$ T cell responses associated with control of viremia. *Science.* 1997;278:1447-1450.