

BD Anti-BrdU (B44)

Monoclonal Antibodies Detecting Cell Proliferation and Activation

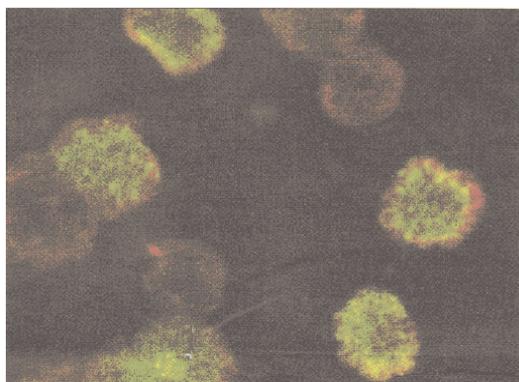
Form	Catalog number
Pure	347580
FITC	347583

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

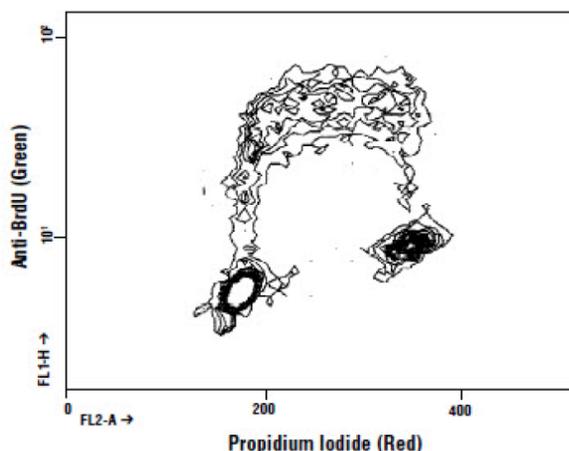
Research Applications

- Research applications[†] include studies of:
- Cells in G1, S, and G2 + M phases of the cell cycle by flow cytometry¹⁻⁶
- Cell proliferation in the presence of cytotoxic drugs^{2,3}
- Sister chromatid exchange using low levels of BrdU⁷

Fluorescence Microscopy



Flow Cytometric Analysis (Linear Fluorescence Intensity)



Description

Specificity

The Anti-BrdU antibody is a uridine derivative that can be incorporated 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}

Antigen distribution

The Anti-BrdU antigen is an analog of thymidine (derivative of uridine) and can be incorporated specifically into DNA in place of thymidine. 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

[†] The published methods in the cited references have not been developed or tested by BD.

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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.

Clone

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

Composition

The Anti-BrdU antibody is composed of mouse IgG1 heavy chains and kappa light chains.

Product configuration

The following are supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
Pure	100	20	50	2.0	25	Gelatin	0.1% Sodium azide
FITC	100	20	50	2.0	25	Gelatin	0.1% Sodium azide

Procedure

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

Labeling Cells with Anti-BrdU and Propidium Iodide (PI) for Flow Cytometric or Microscopic Analysis

Mouse or human cell suspension (see the *Becton Dickinson procedures for preparation of cell suspensions, Monoclonal Antibodies Source Book*, Sections 2.1 and 2.2) or tissue culture cells.

Reagents

- 1X PBS
- Tissue culture medium selected for optimal growth of cells
- 1.0% bovine serum albumin (BSA) (w/v) in PBS
- 0.5% Tween[®] 20 (v/v) plus 1.0% BSA (w/v) in PBS
- 1 mM BrdU (Sigma Chemical Co). Reconstitute in PBS to make a working solution. Aliquots may be frozen for later use.
- **Direct Immunofluorescence Staining:** FITC-conjugated Anti-BrdU (Catalog No. 347583)
- **Indirect Immunofluorescence Staining:** Anti-BrdU (Catalog No. 347580) and FITC-conjugated F(ab')₂ Goat Anti-Mouse IgG (H + L chains) [GAM IgG], (CALTAG Laboratories)
- Propidium iodide stock, 1 mg/mL in PBS. Dilute to 5 µg/mL in PBS for flow cytometric analysis. Dilute to 0.04 µg/mL in PBS for microscopic analysis.
- 2N HCl with 0.5% Triton X-100 (v/v)
- 0.07 N NaOH

- Aqueous ethanol, 70%, –20 °C
- 0.1 M sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$), pH 8.5
- Flo-Texx[®] mounting medium (Lerner Laboratories)

Equipment

- CO₂ incubator, 37 °C
- Low-speed centrifuge with swinging-bucket rotor
- Falcon[®] 12 x 75-mm tubes
- Freezer at –20 °C
- BD FACS™ flow cytometer or fluorescence microscope

Staining with flow cytometric analysis

1. Suspend 1–100 x 10⁶ cells in appropriate tissue culture medium to give optimal conditions for cell growth (DMEM, RPMI, etc). Do not wash cells just prior to incubation with BrdU. This will slow the growth of the cells during the incorporation phase of the procedure. Add BrdU directly to the culture medium to achieve a final concentration of 10 μM. Incubate the cells for 30 minutes in the CO₂ incubator at 37 °C. (The timing of the incubation may be decreased if less BrdU is to be incorporated into the cell population. As little as 2 minutes of incubation can be sufficient to detect DNA synthesis in rapidly growing cells.)
2. Wash the cells twice in 1% BSA/PBS and spin at 500 x g for 15 minutes at room temperature. Resuspend the pellet in 200 μL of 1X PBS on ice.
3. Place 5 mL of 70% ethanol into <glass> test tubes and store at –20 °C until ready for use. Slowly add cells, a few drops at a time, into the ethanol while maintaining a vortex. Incubate on ice for 30 minutes. The cells are now fixed.
4. Centrifuge cells at 500 x g for 10 minutes at 10 °C. Aspirate the supernatant carefully. Loosen pellet by vortexing.
5. Slowly add 1 mL of 2N HCl/Triton X-100 to the cells, a few drops at a time, while maintaining a vortex. Incubate at room temperature for an additional 30 minutes. This denatures the DNA to produce single-stranded molecules.
6. Centrifuge the cells at 500 x g for 10 minutes. Aspirate the supernatant and resuspend in 1 mL of 0.1 M $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$, pH 8.5, to neutralize the acid. (BrdU-labeled cells may be stored at this stage by centrifugation, followed by resuspension in cold 70% ethanol and stored at –20 °C.)
7. Centrifuge the cells at 500 x g for 10 minutes. Aspirate the supernatant and resuspend in 1 mL of 0.5% Tween[®] 20/1% BSA/PBS. Adjust the cell concentration to achieve 1 x 10⁶ cells/test.
8. **For direct immunofluorescence staining**, add 20 μL of Anti-BrdU FITC per 10⁶ cells and incubate for 30 minutes at room temperature. Wash once in 1 mL Tween/BSA/PBS.

For indirect immunofluorescence staining, add 20 μL of Anti-BrdU per 10⁶ cells and incubate for 30 minutes at room temperature. Centrifuge (500 x g for 5 minutes) and resuspend the pellet in 50 μL of 0.5% Tween 20/BSA/PBS solution. Add an appropriate concentration of F(ab')₂ GAM IgG FITC, for example, 1 μg per test or as recommended by the manufacturer. Incubate at room temperature for 30 minutes.

9. Centrifuge cells (500 x g for 5 minutes) and resuspend in 1 mL of 1X PBS containing 5 µg/mL of propidium iodide.
10. Analyze on a BD FACS™ flow cytometer. Laser excitation is at 488 nm.

Staining with microscopic analysis

1. Suspend cells in appropriate tissue culture medium to give optimal conditions for cell growth (DMEM, RPMI, etc). Do not wash cells just prior to incubation with BrdU. This will slow the growth of the cells during the incorporation phase of the procedure. Add BrdU directly to the culture medium to achieve a final concentration of 10 µM. Incubate the cells for 30 minutes in the CO₂ incubator at 37 °C. (The timing of the incubation may be decreased if less BrdU is to be incorporated into the cell population. As little as 2 minutes of incubation may be sufficient to detect DNA synthesis in rapidly growing cells.)
2. Prepare cytocentrifuge slides or smears of the labeled cells.
3. Fix in 70% ethanol for 30 minutes at room temperature.
4. Air dry the slides.
5. Immerse the slides in 0.07 N NaOH for 2 minutes.
6. Immerse the slides in a Coplin jar containing PBS, pH 8.5, to neutralize the base.
7. Mix 20 µL of Anti-BrdU (either FITC-conjugated or unconjugated) with 50 µL of 0.5% Tween[®] 20/PBS.
8. **For direct immunofluorescence staining**, add diluted Anti-BrdU FITC to slide and incubate for 30 minutes in a humidified chamber. Wash with PBS.

For indirect immunofluorescence staining, add diluted unconjugated Anti-BrdU to slide and incubate for 30 minutes in a humidified chamber. Wash with PBS. Then add 50 µL of 0.5% Tween 20/PBS. Add an appropriate concentration of F(ab')₂ GAM IgG FITC, for example, 1 µg per test or as recommended by the manufacturer. Incubate at room temperature for 30 minutes. Wash with PBS.

9. Incubate for 1 minute in 0.04 µg/mL propidium iodide (PI).

NOTE Too much PI at this step causes the red DNA stain to predominate over the green immunofluorescence.

10. Wash the cells with water, dry, and apply coverslip using Flo-Texx mounting medium prior to microscopic examination.

Handling and Storage

Store vials at 2–8 °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

Propidium iodide is a possible mutagen. Avoid contact with the quantity and contents as stated on the label at the time of exposure to skin and mucous membranes.

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{8,9} 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.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent.

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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