

**Monoclonal
Antibodies
Detecting Cell
Proliferation and
Activation**

**Anti-BrdU*
(Bromodeoxyuridine)**

Pure Catalog No. 347580 (7580) 100 Tests
FITC Catalog No. 347583 (7583) 100 Tests

DESCRIPTION

Specificity

Bromodeoxyuridine (BrdU) 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}

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

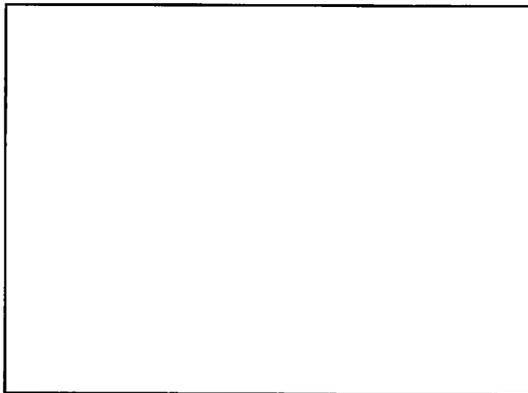
Anti-BrdU is composed of mouse IgG₁ heavy chains and kappa light chains.

NOTE: BrdU 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 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.

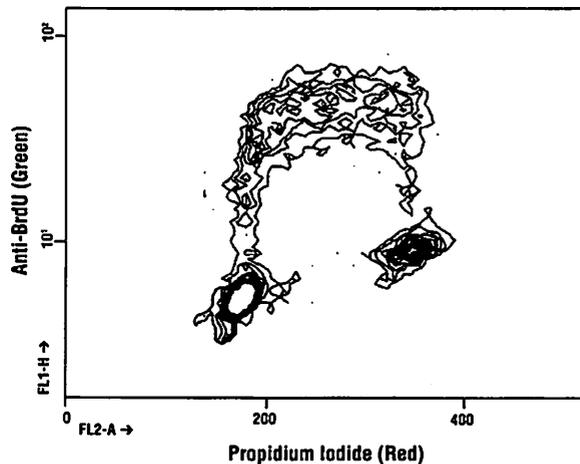
**RESEARCH
APPLICATIONS†**

- Studies of cells in G₁, S, and G₂ + M phases of the cell cycle by flow cytometry¹⁻⁶
- Studies of cell proliferation in the presence of cytotoxic drugs^{2,3}
- Studies of sister chromatid exchange using low levels of BrdU⁷

Fluorescence Microscopy



**Flow Cytometric Analysis
(Linear Fluorescence Intensity)**



* US Patent No. 4,529,700.

† The published methods in the cited references have not been developed or tested by Becton Dickinson.

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

Source Book Section 3.80.1

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 phosphate-buffered saline (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 (Na₂B₄O₇ · 10 H₂O), 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
- FACS[®] brand flow cytometer or fluorescence microscope

Procedure for Staining with Flow Cytometric Analysis

- 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.)
- 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.
- 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.
- Centrifuge cells at 500 x g for 10 minutes at 10°C. Aspirate the supernatant carefully. Loosen pellet by vortexing.
- 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.

- Centrifuge the cells at 500 x g for 10 minutes. Aspirate the supernatant and resuspend in 1 mL of 0.1 M Na₂B₄O₇ · 10 H₂O, 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.)
- 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.
- 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.
- Centrifuge cells (500 x g for 5 minutes) and resuspend in 1 mL of 1X PBS containing 5 µg/mL of propidium iodide.
- Analyze on a FACS brand flow cytometer. Laser excitation is at 488 nm.

Procedure for Staining with Microscopic Analysis

- 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.)
- Prepare cytocentrifuge slides or smears of the labeled cells.
- Fix in 70% ethanol for 30 minutes at room temperature.
- Air dry the slides.
- Immerse the slides in 0.07 N NaOH for 2 minutes.
- Immerse the slides in a Coplin jar containing PBS, pH 8.5, to neutralize the base.
- Mix 20 µL of Anti-BrdU (either FITC-conjugated or unconjugated) with 50 µL of 0.5% Tween 20/PBS.
- 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.
- Incubate for 1 minute in 0.04 µg/mL propidium iodide. NOTE: Too much PI at this step causes the red DNA stain to predominate over the green immunofluorescence.
- Wash the cells with water, dry, and apply coverslip using Flo-Texx mounting medium prior to microscopic exami-

BD Pharmingen™

Purified anti-BrdU
0.1 mg, 0.2 ml, 0.5 mg/ml
Cat: 555627 Lot: 42044
Exp: 2008-02-07 Store at 4°C
Contains <0.1% Sodium Azide
Not intended for diagnostic procedure

BD Biosciences Pharmingen
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For Research Use Only. Not for resale.

Source Book Section 3.80.2

takes ~ 3hrs from #5 to end with 14 samples

ICN 191436

Must count cells and do in suspension → PPE depleted cell arrest in G2 so no longer have equal #s on the dishes

Fix as normally do, but gently vortex cells while adding 70% EtOH. -20°C, 1hr

2nd

0.

1hr

1:50 dilution

650ml 13ml

$$0.5\% = \frac{100\% \times n}{100 \text{ mL}}$$

$n = 0.5 \text{ mL}$
 Tween 20
 into 99.5 mL 1% BSA/PBS

HCl FW = 36.46

$$\left(\frac{36.46 \text{ g}}{\text{mol}} \right) \left(\frac{2 \text{ M}}{\text{L}} \right) \left(\frac{0.05 \text{ L}}{1} \right) = 3.646 \text{ mL HCl}$$

per 50 mL total volume

$$0.5\% \text{ Triton} = \frac{100\% \times n}{50 \text{ mL}} = 250 \mu\text{L Triton X-100}$$

$$\left(\frac{381.37 \text{ g}}{\text{mol}} \right) \left(\frac{0.1 \text{ M}}{\text{L}} \right) \left(\frac{0.1 \text{ L}}{1} \right) = 3.8137 \text{ g per 100 mL}$$

1.90685 g per 50 mL

] $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$

1mM br-dU :

$$\left(\frac{307.1 \text{ g}}{\text{mol}} \right) \left(\frac{0.001 \text{ M}}{\text{L}} \right) \left(\frac{0.01 \text{ L}}{1} \right) = 3.071 \text{ mg / 10 mL PBS}$$

5mg / 16.2 mL

$$20 \mu\text{M} = .02 \text{ mM}$$

$$.02 = \frac{1 \times n}{35 \text{ mL}}$$

700 μL br-dU
 34.3 mL Media

HANDLING AND STORAGE

The monoclonal antibody is supplied as 50 µg purified immunoglobulin in 2.0 mL (25 µg/mL) of phosphate-buffered saline. The FITC conjugate is supplied as 50 µg in 2.0 mL (25 µg/mL). Buffered saline contains gelatin and 0.1% sodium azide. The 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.

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CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of monoclonal antibody is tested for conformance with characteristics of a standard reagent using fluorescence analysis with a FACS brand flow cytometer. Standard procedures are presented in detail in the Becton Dickinson *Monoclonal Antibodies Source Book*.

WARNING

Propidium iodide is a possible mutagen. Avoid contact with skin and mucous membranes. Reagents contain sodium azide. Under aqueous acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions may develop.