Anti-BrdU (B44)

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 drugs2,3
- Sister chromatid exchange using low levels of BrdU7

**RESEARCH APPLICATIONS**

**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 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

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

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

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

a. Volume required to stain 10^6 cells.

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

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.

1. 1X PBS
2. Tissue culture medium selected for optimal growth of cells
3. 1.0% bovine serum albumin (BSA) (w/v) in PBS
4. 0.5% Tween® 20 (v/v) plus 1.0% BSA (w/v) in PBS
5. 1 mM BrdU (Sigma Chemical Co). Reconstitute in PBS to make a working solution. Aliquots may be frozen for later use.

6. 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)

7. 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.

8. 2N HCl with 0.5% Triton X-100 (v/v)
9. 0.07 N NaOH
10. Aqueous ethanol, 70%, -20°C
11. 0.1 M sodium tetraborate (Na2B4O7 · 10 H2O), pH 8.5
12. Flo-Texx® mounting medium (Lerner Laboratories)

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

Staining with flow cytometric analysis

1. Suspend 1–100 x 10^6 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 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.)

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 10^6 cells/test.

8. For direct immunofluorescence staining, add 20 µL of Anti-BrdU FITC per 10^6 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 10^6 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 FACS brand 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. 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°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**

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 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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