For life science research only.
Not for use in diagnostic procedures.

Anti-Bromodeoxyuridine

from mouse IgG_1 (clone: BMC 9318)

Version: 10
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Mouse monoclonal antibody to bromodeoxyuridine
(5-bromo-2′-deoxyuridine-5′-monophosphate), formalin grade.

Cat. No. 11 170 376 001	50 µg 500 µl

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-Bromodeoxyuridine	Solution containing 0.5 ml of phosphate-buffered saline (PBS), pH 7.4, containing 0.09% (w/v) sodium azide and 0.2% (w/v) gelatin for stability.	1 vial, 50 µg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Anti-Bromodeoxyuridine	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Water bath
- Centrifuge
- Cryostat
- Humidified chamber
- Albumin- or gelatin-coated slides
- Cover slips

For flow cytometry

- 5-Bromo-2′-deoxyuridine*
- 70% (v/v) ethanol
- 0.1 M HCl
- 0.5% (v/v) Triton X-100*
- PBS*
- 0.1% (w/v) BSA
- Double-distilled water
- Goat anti-mouse IgG-FITC

For immunohistochemistry

- OCT medium
- Liquid nitrogen
- Absolute methanol
- 2 M HCl
- PBS*
- 0.1% (w/v) BSA
- 0.1 M borate buffer, pH 8.5
- Anti-mouse IgG-peroxidase
- Harris Modified hematoxylin (optional)

1.4. Application

Anti-Bromodeoxyuridine can be used with:
- Cryosections
- Immunochemistry
- Flow cytometry
- Paraffin sections

The monoclonal antibody can also be used to:
- Identify proliferating cells in blood, tissues, and tumors.
- Determine plasma cell labeling indices.

2. How to Use this Product

2.1. Protocols

Flow cytometry

1. To label, pulse cells with 10 µM 5-Bromo-2′-deoxyuridine* for 30 minutes.
   – Harvest cells from culture.

2. Fix cells in 70% (v/v) ethanol at +2 to +8°C for at least 30 minutes.

3. Extract histones by resuspending cells in 1 ml chilled 0.1 M HCl containing 0.5% (v/v) Triton X-100*.
   – Incubate the suspension on ice for 10 minutes.
   – Dilute acid with 5 ml double-distilled water and centrifuge at 200 × *g* for 10 minutes.
   – Resuspend cells in 2 ml double-distilled water.

4. Denature cellular DNA by submerging the cell suspension into a boiling water bath for 10 minutes.
   – Cool suspension immediately by placing it in an ice slurry for several minutes.
   – Wash cells in PBS* containing 0.5% Triton X-100.

5. Resuspend 1 to 2 × 10^6 cells in 100 µl of solution containing approximately 2 µg/ml Anti-Bromodeoxyuridine diluted in PBS containing 0.1% BSA (w/v) (0.2 µg/test).
   – Incubate for 30 minutes at +15 to +25°C.
   – Wash cells with PBS.

6. Resuspend cells in 100 µl of diluted goat anti-mouse IgG-FITC.
   – Wash cells with PBS.

Immunohistochemistry

The following protocol describes the staining of cells labeled with BrdU *in vivo* or *in vitro*.

Preparation of tissue

1. Inject animal with 50 mg BrdU/kg body weight.

2. Sacrifice animal 1 hour later and remove organ or tissue under study.

3. Embed tissue in OCT medium and snap-freeze by immersion into liquid nitrogen.

4. Cut 4 µm frozen sections with a cryostat.

5. Place sections on either albumin- or gelatin-coated slides.

Preparation of cells

Cells grown on cover slips or cytocentrifuge preparations made from cells grown in suspension can be used for Anti-Bromodeoxyuridine staining.

1. Pulse cells with 10 µM BrdU for 60 minutes.

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2. Fix tissue sections or cells on a slide or cover glass by immersing in absolute methanol for 10 minutes at +2 to +8°C.
   - Air dry after removing from fixative.
   - Store slides at −15 to −25°C in a sealed box or rehydrated to prepare for the assay procedure.
   - To rehydrate, immerse in PBS for 3 minutes.

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3. Denature DNA by incubating the slides in 2 M HCl for 60 minutes at +37°C.

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4. Neutralize the acid by immersing the slides in 0.1 M borate buffer, pH 8.5.
   - Change the buffer twice over a 10 minute period.

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5. Wash slides with PBS, changing the solution three times over a 10 minute period.

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6. Place slides in a humidified chamber such as a sealed plastic box layered with wet paper towels.
   - Cover cells with 150 to 300 µl of a solution containing approximately 6 µg/ml Anti-Bromodeoxyuridine diluted in PBS with 0.1% BSA.
   - Incubate for 60 minutes at +15 to +25°C.

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7. Wash slides with PBS, changing the solution three times over a 10 minute period.

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8. Apply an optimum dilution of a second antibody conjugate, such as anti-mouse IgG-peroxidase.
   - Incubate, wash, and perform detection with a substrate that produces an insoluble product.

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9. After detection, counterstain with Harris Modified hematoxylin if desired.
   - Slides can then be dehydrated and mounted.

2.2. Parameters

Purity

≥90%
Determined by HPLC.

Specificity

- The antibody specifically binds to bromodeoxyuridine and cross-reacts with iodouridine (10%).
- Anti-Bromodeoxyuridine does not cross-react with fluorodeoxyuridine, nor with any endogenous cellular components such as thymidine or uridine.

Working Concentration

Application	Concentration
Flow cytometry	0.2 µg/100 µl/10^6 cells
Immunohistochemistry	6 µg/ml

The above mentioned concentration values are guidelines only. The optimum concentration must be determined by the investigator. To maintain stability of the antibody, dilute in PBS, pH 7.4 containing 0.1% BSA.

3. Additional Information on this Product

3.1. Test Principle

During apoptosis, caspases cleave intracellular proteins; the corresponding caspase cleaving sites are formalin resistant. Formalin-grade antibodies recognize cleavage sites in apoptotic cells that are not accessible in normal cells, which allows for determination of caspase activity, even in formalin-fixed, paraffin-embedded tissue.
Bromodeoxyuridine (BrdU) is a thymidine analog and is specifically incorporated into DNA during DNA synthesis. Anti-Bromodeoxyuridine is used to identify cells that have incorporated BrdU. This immunological detection scheme has several advantages over the use of radioactive thymidine incorporation for identifying cells undergoing replication:

- Labeling and detection can be performed the same day instead of waiting several days, as required for autoradiography of tritium-labeled cells, and the necessity of using multiple specimens for obtaining the optimal exposure time is eliminated.
- In addition, anti-bromodeoxyuridine staining with flow cytometric analysis allows multiple parameters to be evaluated simultaneously.

Preparation

1. BALB/c mice were immunized with a bromodeoxyuridine-bovine serum albumin conjugate.
2. Lymphocytes isolated from the spleen were fused with Ag8.653 myeloma cells to create the BMC 9318 clone.
3. The antibody was purified by ion-exchange chromatography.

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
5-Bromo-2'-deoxyuridine	1 g	10 280 879 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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