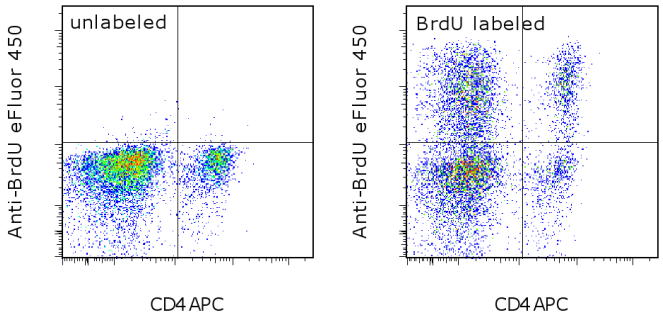
eBioscience™ BrdU Staining Kit for Flow Cytometry eFluor™ 450

Catalog Number: 8848-6600
Also known as: 5-bromodeoxyuridine
RUO: For Research Use Only. Not for use in diagnostic procedures.



Anti-CD3/CD28-stimulated mouse splenocytes either unlabeled (left) or labeled with BrdU (right) were intracellularly stained with Anti-Mouse CD4 APC (cat. 17-0041) and Anti-BrdU eFluor® 450 using the BrdU Staining Kit for Flow Cytometry eFluor® 450 and protocol. Total viable cells were used for analysis.

Product Information

Contents: eBioscience™ BrdU Staining Kit for Flow Cytometry eFluor™ 450
REF **Catalog Number:** 8848-6600
Handling Conditions: Use within 6 months of opening or by date indicated on the bottle

Temperature Limitation: Store at 2-8°C, except BrdU and Dnase I, which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide and formaldehyde

Description

The eBioscience BrdU Staining Kit for Flow Cytometry contains the necessary reagents and buffers for identifying and examining proliferating cells of mammalian species by flow cytometric analysis. Cycling cells are incubated with 5-bromo-2'-deoxyuridine (BrdU), a synthetic analog of thymidine which incorporates into newly synthesized genomic DNA during the S-phase of mitosis. Following DNA denaturation, the cells are stained for BrdU incorporation along with any other cell surface and/or intracellular targets of interest.

This kit is optimized to achieve brighter staining compared to traditional protocols.

Components

BrdU (cat. 00-4440-51A): 5 x 1 mL sterile vials; store at less than or equal to -70°C.
DNase I (cat. 00-4425-10): 10 x 0.3 mL vials; store at less than or equal to -70°C.
Anti-BrdU eFluor® 450 Antibody (clone BU20A) (cat. 48-5071-42): 1 x 100 test vial; store at 2-8°C.
BrdU Staining Buffer Concentrate (4X) (cat. 00-5515-43): 1 x 30 mL bottle; store at 2-8°C.
Fixation/Permeabilization Diluent (cat. 00-5223-56): 1 x 100 mL bottle; store at 2-8°C.

Applications Reported

The BrdU Staining Kit for Flow Cytometry eFluor® 450 has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

The BrdU Staining Kit for Flow Cytometry eFluor® 450 has been tested by flow cytometric analysis of BrdU-labeled mouse splenocytes. The BU20A antibody can be used at 5 µL (0.25 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test.

eFluor® 450 is a replacement for Pacific Blue®. eFluor® 450 emits at 456 nm and is excited with the Violet laser (405 nm). Please make sure that your instrument is capable of detecting this fluorochome.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

eBioscience™ BrdU Staining Kit for Flow Cytometry eFluor™ 450

Catalog Number: 8848-6600
Also known as: 5-bromodeoxyuridine
RUO: For Research Use Only. Not for use in diagnostic procedures.

References

Magaud JP, Sargent I, Clarke PJ, Ffrench M, Rimokh R, Mason DY. Double immunocytochemical labeling of cell and tissue samples with monoclonal anti-bromodeoxyuridine. J Histochem Cytochem. 1989 Oct;37(10):1517-27. (**Bu20a**, FC)

Beisker W, Dolbeare F, Gray JW. An improved immunocytochemical procedure for high-sensitivity detection of incorpo-rated bromodeoxyuridine. Cytometry. 1987;8:235.

Gratzner, D.F., H.G. Pallavicini and M.G. Gray. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine.1983 Proc. Natl. Acad. Sci. USA 80:5573.

Dolbeare F, Gratzner HG, Pallavicini MG, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. 1983 Proc Natl Acad Sci USA.80:5573.

Related Products

12-5699 eBioscience™ Anti-Human Ki-67 PE (20Raj1)
16-0031 eBioscience™ Anti-Mouse CD3e Functional Grade Purified (145-2C11)
16-0281 eBioscience™ Anti-Mouse CD28 Functional Grade Purified (37.51)
17-0041 eBioscience™ Anti-Mouse CD4 APC (GK1.5)
48-4714 eBioscience™ Mouse IgG1 K Isotype Control eFluor™ 450 (P3.6.2.8.1)
65-0865 eBioscience™ Fixable Viability Dye eFluor™ 780

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

invitrogen

BrdU Staining Kit for Flow Cytometry

Introduction

The Thermo Fisher BrdU Staining Kit for Flow Cytometry contains the necessary reagents and buffers for identifying and examining proliferating cells by flow cytometric analysis. Cycling cells are incubated with 5-bromo-2'-deoxyuridine (BrdU), a synthetic analog of thymidine which incorporates into newly synthesized genomic DNA during the S-phase of mitosis. Following DNA denaturation, the cells are stained for BrdU incorporation along with any other cell surface and/or intracellular targets of interest.

General Notes

Please use caution when handling. Product contains formaldehyde.

Protocol

Materials Provided

- BrdU (32.5 mM, 10 mg/mL): 5 x 1 mL sterile vials; store at less than or equal to -70°C. Avoid multiple freeze-thaws. Vial should be opened under sterile conditions.
- DNase I (1 mg/mL; 0.3 mg/vial): 10 x 0.3 mL vials; store at less than or equal to -70°C. Each vial can be used to treat 10 samples. Avoid multiple freeze-thaws.
- Anti-BrdU Antibody (clone BU20A), fluorochrome-conjugated: 1 x 100 test vial; store at 2-8°C.
- BrdU Staining Buffer Concentrate (4X): 1 x 30 mL bottle; store at 2-8°C. This buffer contains formaldehyde. Please handle appropriately.
- Fixation/Permeabilization Diluent: 1 x 100 mL bottle; store at 2-8°C.

Other Materials Needed

- Sterile 1X PBS
- Flow Cytometry Staining Buffer Thermo Fisher (Cat. No. 00-4222)
- 12 x 75 mm round bottom test tubes
- Optional:
  - Primary antibodies (directly conjugated)
  - Fixable Viability Dye (FVD) eFluor™ 450 (Thermo Fisher Cat. No. 65-0863), eFluor™ 660 (Thermo Fisher Cat. No. 65-0864), eFluor™ 780 (Thermo Fisher Cat. No. 65-0865), eFluor™ 506 (Thermo Fisher Cat. No. 65-0866), eFluor™ 520 (Thermo Fisher Cat. No. 65-0867) and eFluor™ 455UV (Thermo Fisher Cat. No. 65-0868)

Note: The antibodies used for surface staining can be added after BrdU and Fixable Viability Dye labeling (but before fixation). Alternatively if the antibody(s) is known to recognize a formaldehyde- fixed epitope, it can be added concurrently with the BrdU antibody.

BrdU Staining Buffer Working Solution Preparation

- Prepare fresh 1X BrdU Staining Buffer working solution by diluting BrdU Staining Buffer Concentrate (1 part) with Fixation/Permeabilization Diluent (3 parts). Mix by gentle inversion, do not vortex. You will need 1 mL of the 1X BrdU Staining Buffer working solution for each sample. Use caution and handle appropriately as the buffer contains formaldehyde.

Experimental Procedure

Step 1: In vitro labeling of 10^5 to 10^8 dividing cells with 10 µM BrdU for 45 minutes at 37°C.

a) Under sterile conditions thaw BrdU on ice and dilute to a working concentration of 1 mM with sterile 1X PBS.

b) Add 10 µM BrdU to each sample. (For example, add 10 µL of 1 mM BrdU directly to every milliliter of tissue culture medium.)

c) Incubate your cells long enough to allow incorporation of BrdU. The timing will be dependent on your culture conditions (e.g., stimulants used) and the proliferation kinetics of your cells. Therefore the incubation time will need to be determined empirically. After the incubation, harvest the cells.

d) Wash cells by adding 2 mL of Flow Cytometry Staining Buffer (or azide-free PBS if proceeding to Step 2) and then centrifuge at 300-400 xg for 5 minutes at room temperature. Discard the supernatant.

Step 2: [Optional] Stain with Fixable Viability Dye (FVD) to label dead cells before fixation.

Note: Allow the vial of Fixable Viability Dye to equilibrate to room temperature before opening. The dye must be used with azide-free PBS. For consistent staining of cells in tubes, do not stain in less than 0.5 mL. Please refer to the Thermo Fisher website Best Protocols "Viability Staining Protocol, Protocol C: Staining Dead Cells with Thermo Fisher Fixable Viability eFluor™ Dyes" for additional information. (Proceed to Step 3 if a FVD will not be used.)

a) Wash cells one additional time with 2 mL of azide-free PBS, as described in Step 1d.

b) Resuspend cells at 1-10x10^6 cells/mL in azide-free PBS.
c) Add 1 µL of Fixable Viability Dye per 1 mL of cells and vortex immediately.
d) Incubate for 30 minutes at 2-8°C in the dark.
e) Wash cells 1-2 times with Flow Cytometry Staining Buffer, as described in Step 1d.
f) Resuspend cells at 1-10x10^6 cells/mL in Flow Cytometry Staining Buffer.

Step 3: [Optional] Stain cell surface antigen(s).

Note: For additional information, please refer to the Thermo Fisher website Best Protocols "Staining Cell Surface Antigens for Flow Cytometry." (Proceed to Step 4 if cell surface antigens will not be examined or if the antibody(s) is known to recognize a formaldehyde-fixed epitope.)

a) Aliquot 50 µL of cell suspension to each tube or well. The cell number should be determined empirically but can range from 10^5 to 10^8 cells/test per tube.
b) Add the recommended amount (refer to the Technical Data Sheet for each product) of each fluorochrome-conjugated primary antibody(s) in an appropriate volume of Flow Cytometry Staining Buffer such that the final staining volume is 100 µL. (For example, add 50 µL of an antibody mix to 50 µL of cells.) Mix gently.
c) Incubate for at least 30 minutes at 2-8°C in the dark.
d) Wash the cells twice with Flow Cytometry Staining Buffer, as described in Step 1d.

Step 4: Fix cells and intracellularly stain with Anti-BrdU.

a) Thaw DNase I solution on ice. Once thawed, prepare a working solution of DNase I by adding 300 µL of the DNase I solution to 700 µL of Flow Cytometry Staining Buffer and mix gently. Store on ice until ready for use in Step 4f, below.
b) If cells were not stained in Steps 2 or 3, aliquot 100 µL of cell suspension to each tube. The cell number should be determined empirically but can range from 10^5 to 10^8 cells/tube.
c) Gently resuspend the cells from Step 2f, Step 3d, or Step 4b, by pulse-vortexing once. This resuspension step is critical before the addition of the freshly prepared 1X BrdU Staining Buffer working solution.
d) Add 1 mL of freshly prepared 1X BrdU Staining Buffer working solution and mix gently. Incubate for 15 minutes at room temperature in the dark. Incubations may go longer (up to 14 hours) but should be determined empirically for each cell type.
e) Wash cells twice with Flow Cytometry Staining Buffer, as described in Step 1d.
f) Add 100 µL of the DNase I working solution that was prepared in Step 4a to each sample. Incubate for 1 hour at 37°C in the dark.
g) Wash cells twice with Flow Cytometry Staining Buffer, as described in Step 1d.
h) Add 5 µL of Anti-BrdU fluorochrome-conjugated antibody per sample. Mix and incubate for 20-30 minutes at room temperature in the dark.

   Note: Antibodies against intracellular antigens or surface antigens not stained in Step 3 may be added here. The antibodies used for surface staining at this step must recognize a fixed epitope. If an antibody only recognizes a native epitope or if this information is unknown, we recommend surface staining at Step 3.
i) Wash cells twice with Flow Cytometry Staining Buffer, as described in Step 1d.

Step 5: Acquire data on a flow cytometer.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  a) Product FAQs
  b) Software, patches, and updates
- Order and web support
- Product documentation, including:
  a) User guides, manuals, and protocols
  b) Certificates of Analysis
  c) Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288