eBioscience™ BrdU Staining Buffer Set for Flow Cytometry

Catalog Number: 00-5525
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 (cat. 8848-6600) and protocol. Total viable cells were used for analysis.

Product Information

Contents: eBioscience™ BrdU Staining Buffer Set for Flow Cytometry
REF **Catalog Number:** 00-5525
Handling Conditions: Use within 6 months of opening or by date indicated on the bottle

Temperature Limitation: Store at 2-8°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide and formaldehyde

Description

The BrdU Staining Buffer Set contains the optimized buffers needed for optimal BrdU staining with the BU20A monoclonal antibody for flow cytometric analysis. This set in combination with BrdU and DNase I steps allows for detection of BrdU pulse-labeled cells thereby enabling the examination of proliferating cells. 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.

Components

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

BrdU Staining Buffer Set has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

BrdU Staining Buffer Set has been tested by flow cytometric analysis of BrdU-labeled mouse splenocytes using the attached protocol.

Related Products

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)
48-5071 eBioscience™ Anti-BrdU eFluor™ 450 (BU20A)
65-0865 eBioscience™ Fixable Viability Dye eFluor™ 780
8848-6600 eBioscience™ BrdU Staining Kit for Flow Cytometry eFluor™ 450

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invitrogen

BrdU Staining Buffer Set Protocol

Introduction

The 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 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

- BrdU
- DNase I
- Anti-BrdU Antibody (clone BU20A), fluorochrome-conjugated
- Sterile 1X PBS
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 12 x 75 mm round bottom test tubes
- Optional:
  - Primary antibodies (directly conjugated)
  - Fixable Viability Dye (FVD) eFluor™ 450 (Cat. No. 65-0863), eFluor™ 660 (Cat. No. 65-0864), eFluor™ 780 (Cat. No. 65-0865), eFluor™ 506 (Cat. No. 65-0866), eFluor™ 520 (Cat. No. 65-0867) and eFluor™ 455UV (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 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 and stain cells 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.

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

Limited product warranty

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