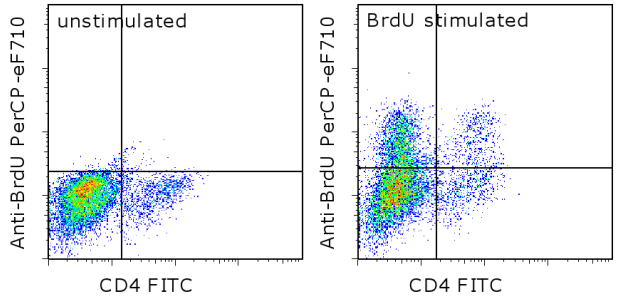
Anti-BrdU PerCP-eFluor® 710

Catalog Number: 46-5071
Also known as: 5-bromodeoxyuridine
RUO: For Research Use Only. Not for use in diagnostic procedures.



Mouse splenocytes either left unstimulated (left) or stimulated with Anti-Mouse CD3/CD28 Functional Grade Purified antibodies (cat. 16-0031, 16-0281) (right) were labeled with BrdU and then stained with Anti-Mouse CD4 FITC (cat. 11-0041) followed by fixation and permeabilization using the Foxp3/Transcription Buffer Set (cat. 00-5523) and protocol. The cells were then stained with Anti-BrdU PerCP-eFluor® 710. Total viable cells were used for analysis.

Product Information

Contents: Anti-BrdU PerCP-eFluor® 710
REF **Catalog Number:** 46-5071
Clone: BU20A
Concentration: 5 uL (0.06 ug)/test
Host/Isotype: Mouse IgG1, kappa

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer
Temperature Limitation: Store at 2-8°C. Do not freeze. Light-sensitive material.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide

Description

This Bu20a monoclonal antibody reacts with 5-bromodeoxyuridine (BrdU). BrdU is a derivative of uridine that can be incorporated into DNA in place of thymidine during the S-phase of the cell cycle. Anti-BrdU can then be used to identify cells that have undergone DNA synthesis during BrdU treatment.

For staining for flow cytometric analysis, we recommend the use of the BrdU Staining Buffer Set (cat. 00-5525) and protocol.

Applications Reported

This BU20A antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This BU20A antibody has been pre-titrated and tested by flow cytometric analysis of BrdU-pulsed mouse splenocytes using the Foxp3/Transcription Factor Buffer Set (cat. 00-5523) and protocol or the BrdU Staining Buffer Set (cat. 00-5525) and protocol. This can be used at 5 µL (0.06 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.

PerCP-eFluor® 710 can be used in place of PE-Cy5, PE-Cy5.5 or PerCP-Cy5.5. PerCP-eFluor® 710 emits at 710 nm and is excited with the blue laser (488 nm). Please make sure that your instrument is capable of detecting this fluorochrome. For a filter configuration, we recommend using the 685 LP dichroic mirror and 710/40 band pass filter, however the 695/40 band pass filter is an acceptable alternative.

Our testing indicates that PerCP-eFluor® 710 conjugated antibodies are stable when stained samples are exposed to freshly prepared 2% formaldehyde overnight at 4°C, but please evaluate for alternative fixation protocols.

Click here or contact eBioscience Technical Support for more information on eFluor™ Organic Dyes including PerCP-eFluor® 710.

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BrdU labeling and staining with the Anti-BrdU antibody:
1. Label dividing cells with 10 µM BrdU for 45 min at 37°C.
2. Following the incubation, harvest the cells and wash once with 1X PBS.
3. Stain surface molecules according to the Surface Staining Protocol.
4. Wash in cold Flow Cytometry Staining Buffer or 1X PBS.
5. Resuspend the cell pellet by pulse vortexing. Then add 1 ml of freshly prepared Foxp3 Fixation/Permeabilization Buffer (cat. 00-5521) to each sample. Pulse vortex again.
6. Incubate for 30 to 60 minutes at 4°C in the dark.
7. Wash once with cold Flow Cytometry Staining Buffer followed by centrifugation. Decant the supernatant.
8. Resuspend the cell pellet with 100 µL Flow Cytometry Staining Buffer containing 30 µg of Dnase I.
9. Incubate for 1 hr at 37°C and then wash.
10. Stain cells with anti-BrdU antibody for 30 min to 1 hr and then wash.
11. Analyze the samples.

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

00-4222 Flow Cytometry Staining Buffer
00-5523 Foxp3 / Transcription Factor Staining Buffer Set
00-5525 BrdU Staining Buffer Set
11-0041 Anti-Mouse CD4 FITC (GK1.5)
16-0031 Anti-Mouse CD3e Functional Grade Purified (145-2C11)
16-0281 Anti-Mouse CD28 Functional Grade Purified (37.51)
65-0865 Fixable Viability Dye eFluor® 780
8846-6600 BrdU Staining Kit for Flow Cytometry PerCP-eFluor® 710

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