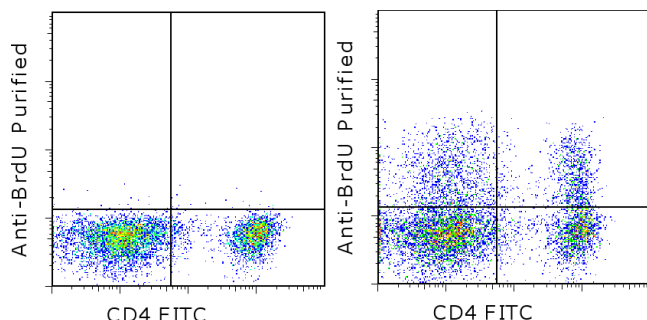


Anti-BrdU Purified

Catalog Number: 14-5071

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 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 Staining Buffer Set (cat. 00-5523) and then stained with 0.5 μ g of Anti-BrdU Purified followed by F(ab')₂ Anti-Mouse IgG PE (cat. 12-4012). Total viable cells were used for analysis.

Product Information

Contents: Anti-BrdU Purified
Catalog Number: 14-5071
Clone: BU20A
Concentration: 0.5 mg/mL
Host/Isotype: Mouse IgG1, kappa

REF

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer
Temperature Limitation: Store at 2-8°C.



Batch Code: Refer to vial

Use By: Refer to vial

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 and immunohistology staining of frozen tissue sections.

Applications Tested

This Bu20A antibody has been tested by flow cytometric analysis of BrdU-labeled mouse splenocytes. This can be used at less than or equal to 1 μ g per test. A test is defined as the amount (μ g) of antibody that will stain a cell sample in a final volume of 100 μ L. Cell number should be determined empirically but can range from 10⁵ to 10⁸ cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

BrdU labeling and staining with anti-BrdU antibody:

1. Label dividing cells with 10 μ M 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 solution (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 by adding 2 ml 1X Permeabilization Buffer (made from 10X Permeabilization Buffer included in the Foxp3 buffer Set) followed by centrifugation. Decant the supernatant.
8. Resuspend the cell pellet with 100 μ L Flow Cytometry Staining Buffer containing 30 μ g of DNase I.

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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. If necessary, stain with secondary antibody for 30 min and then wash.
12. 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 incorporated 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

00-5523 Foxp3 / Transcription Factor Staining Buffer Set
00-5525 BrdU Staining Buffer Set
11-0041 Anti-Mouse CD4 FITC (GK1.5)
12-4010 F(ab')₂ Anti-Mouse IgG PE (polyclonal)
14-4714 Mouse IgG1 K Isotype Control Purified (P3.6.2.8.1)
16-0031 Anti-Mouse CD3e Functional Grade Purified (145-2C11)
16-0281 Anti-Mouse CD28 Functional Grade Purified (37.51)