Technical Data Sheet

PerCP-Cy™5.5 Mouse anti-BrdU

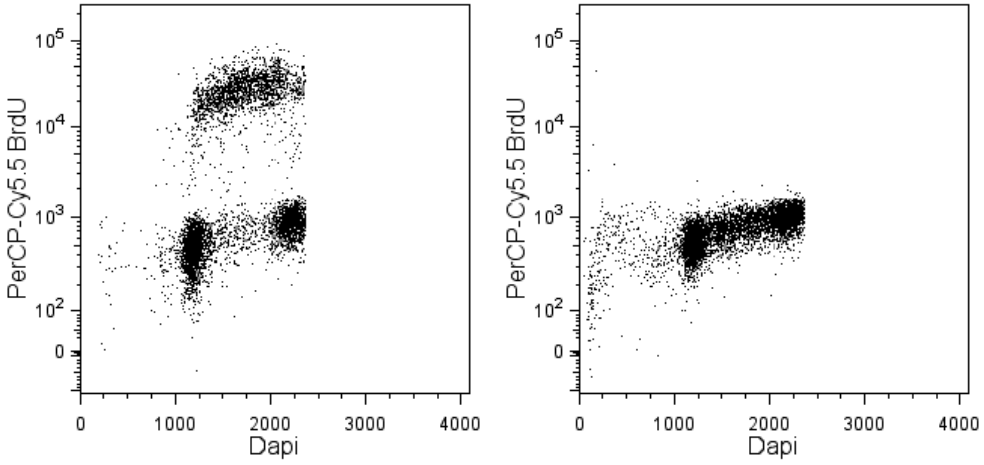
Product Information

Material Number:	**560809**
Alternate Name:	5-bromo-2'-deoxyuridine, 5-Bromouracil deoxyriboside, BUdR
Size:	50 tests
Vol. per Test:	5 µl
Clone:	3D4
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Bromodeoxyuridine (BrdU) is an analog of thymidine that can be incorporated into newly synthesized DNA by cells entering and progressing thru the DNA synthesis (S) phase of the cell cycle. The amount of BrdU that gets incorporated is dependent upon the amount of time that the cells are exposed to BrdU (pulse time), the rate of cell division, and whether the cells are in early, mid, or late S phase. Detection of incorporated BrdU allows the investigator to identify cycling cells in an asynchronous cell population and to determine cell cycle kinetics.

The 3D4 monoclonal antibody reacts with BrdU, but not other nucleotides, in single-stranded DNA. Random cleavage (nicking) of cellular DNA with DNase I permits the binding of the antibody to incorporated BrdU.



Flow cytometric analysis of DNA synthesis by TK-1 cells using PerCP-Cy5.5 anti-BrdU antibody. *TK-1 cells were either pulsed with 50 µM BrdU for 1 hour (left panel) or were not pulsed (right panel). Staining was performed using the procedure from the BD Pharmingen™ FITC BrdU Flow Kit (Cat. No. 559619). The permeabilized cells were stained with the PerCP-Cy5.5 anti-BrdU antibody followed by the DNA-specific dye, DAPI dihydrochloride at 1 µg/ml (Sigma, Cat. No. D9542). Two-color flow cytometric dot plots showing the correlated expression patterns of DAPI vs BrdU were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed with doublet discrimination using a BD™ LSRII System.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Please see the detailed protocol at http://www.bdbiosciences.com/resources/index.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
550891	Bromodeoxyuridine (BrdU)	25 mg	(none)

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Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
3. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
4. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
8. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
9. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
11. All other brands are trademarks of their respective owners.

References

Dolbeare F, Gratzner H, Pallavicini MG, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. *Proc Natl Acad Sci U S A.* 1983; 80(18):5573-5577. (Methodology: Flow cytometr)

Keren DF, Hanson CA, Hurtubise PE, ed. *Flow Cytometry and Clinical Diagnosis.* Chicago: American Society of Clinical Pathologists Press; 1994:1-676. (Methodology: Flow cytometr)

Miltenburger HG, Sachse G, Schliermann M. S-phase cell detection with a monoclonal antibody. *Dev Biol Stand.* 1987; 66:91-99. (Clone-specific: Immunofluorescenc)

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