

Technical Data Sheet

V450 Mouse anti-BrdU

Product Information

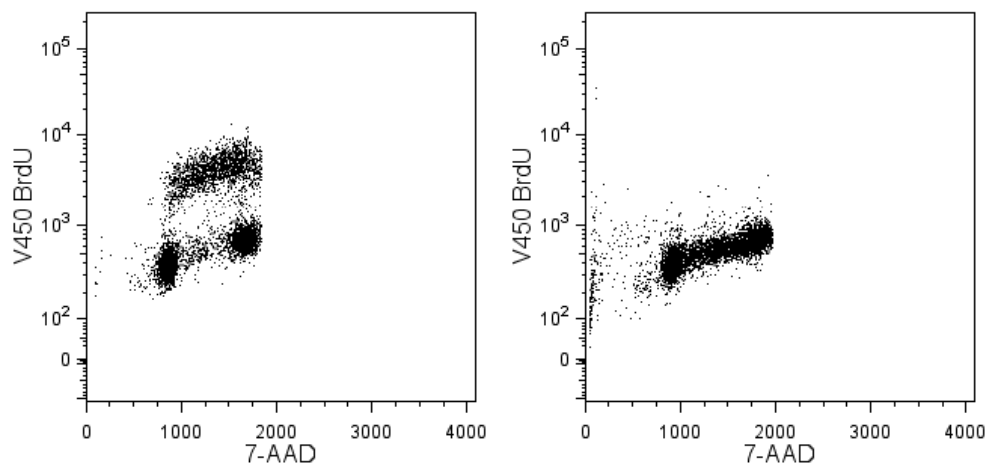
Material Number:	560810
Alternate Name:	5-bromo-2'-deoxyuridine, 5-Bromouracil deoxyriboside, BUdR
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	3D4
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer 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.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex Max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Flow cytometric analysis of DNA synthesis by TK-1 cells using BD Horizon™ V450 anti-BrdU antibody (Cat. No. 560810). 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 BD Horizon™ V450 anti-BrdU antibody followed by the DNA-specific dye, 7-AAD (7-AAD Staining Solution, Cat. No. 559925). Two-color flow cytometric dot plots showing the correlated expression patterns of 7-AAD 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™ LSR II 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 BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

Please see the detailed protocol at http://www.bdbiosciences.com/support/resources/protocols/brdu_detection.jsp.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
550891	Bromodeoxyuridine (BrdU)	25 mg	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554714	BD Cytotfix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
561651	Permeabilization Buffer	10 ml	(none)
559925	7-AAD	2.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. 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.
4. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

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 cytometry)

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

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