Technical Data Sheet

Alexa Fluor® 555 Mouse anti-BrdU

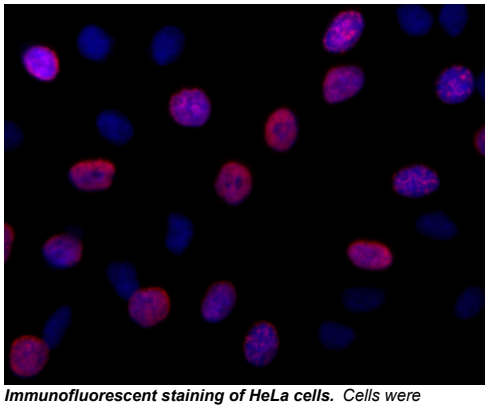
Product Information

Material Number:	**560210**
Alternate Name:	5-bromo-2'-deoxyuridine, 5-Bromouracil deoxyriboside, BUdR
Size:	100 tests
Vol. per Test:	5 µl
Clone:	3D4
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

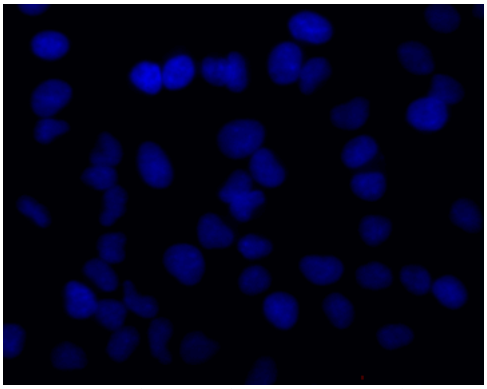
Description

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 vells 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.



Immunofluorescent staining of HeLa cells. *Cells were seeded in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight culture, the cells were loaded with 20 µM BrdU for 1 hour at 37°C. After treatment, the cells were fixed, permeabilized, DNAse treated, stained with Alexa Fluor® 555 Mouse anti-BrdU (pseudo colored red), and counter stained with Hoechst 33342 (pseudo colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 confocal imager with a 20x objective and merged using BD AttoVision™ software.*



Negative control for immunofluorescent staining of HeLa cells. *Cells were cultured as in the left image, but without BrdU loading. They were fixed, permeabilized, DNAse treated, stained with Alexa Fluor® 555 Mouse anti-BrdU (pseudo colored red), and counter stained with Hoechst 33342 (pseudo colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 confocal imager with a 20x objective and merged using BD AttoVision™ software.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 555 under optimum conditions, and unreacted Alexa Fluor® 555 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

Cells are pulse labeled with BrdU, fixed to maintain BrdU loading, and permeabilized for intranuclear staining of the incorporated BrdU. DNase I is used to randomly cleave the DNA, allowing the anti-BrdU monoclonal antibody to bind to the incorporated BrdU.

Materials

- Adherent cell culture growing in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219)
- 37°C incubator
- BrdU (Cat. No. 550891) diluted to 10-100 µM in tissue culture medium, prepare enough to use at 50 µl per well (see step 1 of procedure)

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- BD Cytofix™ fixation buffer (Cat. No. 554655), warmed to 37°C
- BD™ Phosflow Perm Buffer III (Cat. No. 558050), at -20°C
- Phosphate-buffered saline solution (PBS), at room temperature
- BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), at 4°C
- 300 μg/ml sterile solution of DNase I (preferred, Sigma-Aldrich Cat. No. D4513) in PBS, prepare enough to use at 50 μl per well
- Alexa Fluor® 555 Mouse anti-BrdU monoclonal antibody diluted 1:10 in BD Pharmingen™ Stain Buffer (FBS), prepare enough to use at 50 μl per well. If additional antibodies are to be used, they should be added so that all antibodies are included in the 50 μl per well.
- 2 μg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in PBS, prepare enough to use at 100 μl per well
- Fluorescence imaging instrument capable of exciting Alexa Fluor® 555 and Hoechst 33342, such as the BD Pathway™ Bioimaging System

Procedure

1. Pulse the adherent cell cultures by adding 50 μl of the diluted BrdU to each well and incubating for 1-2 hrs at 37°C. Omit the BrdU from the wells that will be used as negative controls for the BrdU staining. In all subsequent steps, the BrdU-loaded and control cells should be processed in parallel.
2. Remove the medium from the wells, and fix the cells by adding 100 μl of the pre-warmed fixation buffer to each well and incubating for 10 - 20 minutes at room temperature.
3. Remove the fixative from the wells, and wash the wells twice with 100 μl of PBS.
4. Remove the PBS, and permeabilize the cells by adding 100 μl of the ice-cold BD™ Phosflow Perm Buffer III to each well and incubating for 5 - 10 minutes at room temperature.
5. Remove the Perm Buffer III from the wells, and wash the wells twice with 100 μl of PBS.
6. OPTIONAL: Remove the PBS, and block the cells by adding 100 μl of the BD Pharmingen™ Stain Buffer (FBS) to each well and incubating for 15 - 30 minutes at room temperature.
7. Remove the BD Pharmingen™ Stain Buffer (FBS), and denature the cells' DNA by adding 50 μl of the sterile DNase I solution to each well and incubating for 1 hour at 37°C.
8. Remove the DNase I solution from the wells, and wash the wells once with 100 μl of PBS.
9. Remove the PBS, and stain the cells by adding 50 μl of the diluted antibody solution to each well and incubating for 1 hour at room temperature.
10. Remove the antibody solution, and wash the wells twice with 100 μl of PBS.
11. Remove the PBS, and counter-stain the nuclei by adding 100 μl of the Hoechst 33342 solution to each well at least 15 minutes before imaging.
12. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ cell analyzers are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	548/20	570LP	Fura/FITC
BD Pathway 435	543/22	593/40	FF562

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
550891	Bromodeoxyuridine (BrdU)	25 mg	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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