BrdU-Mouse Monoclonal Antibodies-Clone MoBU-1

Table 1. Contents and storage information.

Material	Conjugate	Amount	Contents	Storage*
BrdU-Mouse Monoclonal Antibodies-Clone MoBU-1	Pacific Blue™ (B35129)	500 µL	Antibody solution in phosphate buffered saline (PBS), pH 7.2, containing 5 mM sodium azide.	• 2–6°C • Protect from light
	Alexa Fluor® 488 (B35139)			
	Alexa Fluor® 647 (B35140)			
	Unconjugated (B35141)			
*When stored as directed, the product is stable for 1 year.				
Number of assays: Sufficient material is supplied for approximately 100 assays.				
Approximate fluorescence excitation/emission maxima: Pacific Blue™ conjugate: 416/451 in nm; Alexa Fluor® 488 conjugate: 495/518 in nm; Alexa Fluor® 647 conjugate: 650/665 in nm.				

Introduction

The thymidine analog 5-bromo-2-deoxyuridine (BrdU) is a common reagent used for cell proliferation assays[1, 2] and for the detection of apoptotic cells.[3] BrdU is a uridine derivative and a structural analog of thymidine, and it can be incorporated into DNA during the synthesis-phase of the cell cycle as a substitute for thymidine, thereby serving as a marker for proliferation. Cells marked by BrdU incorporation may be detected by fluorescently labeled anti-BrdU antibodies.

The anti-BrdU mouse monoclonal antibody MoBU-1 readily detects BrdU incorporated into DNA. Cells can be pulse-labeled with BrdU, and then analyzed with the antibody clone MoBU-1 against BrdU to determine the proportion of proliferating cells during a given interval.

An advantage of the MoBU-1 clone is that is does not cross-react with the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU), which is detected via click chemistry.[5, 6] Traditionally, the dual pulse method employs BrdU immunocytochemistry and ^{3}H-thymidine radiography, or it combines BrdU with iododeoxyuridine (IdU) or chlorodeoxyuridine (CldU), using multiple BrdU antibodies of different clones that cross-react with IdU and CldU for detection.

Combining BrdU detection with the antibody clone MoBU-1 and EdU detection via click chemistry simplifies dual pulse labeling. Using sequential pulses of the thymidine analogs EdU and BrdU, BrdU- and EdU-labeled cells can easily and reliably be distinguished by flow cytometry. This dual pulse method uses anti-BrdU clone MoBU-1 for the detection of the incorporated BrdU, which shows no cross reactivity with EdU. This is combined with click-chemistry detection of the incorporated EdU, which is bio-orthogonal and does not react with the incorporated BrdU.

Before Starting

Materials Required but Not Provided

- BrdU
- Buffers such as Phosphate Buffered Saline (PBS)
- Cells and culture media
- Reagents for cell fixation and DNA denaturation

Staining Conditions

These reagents were optimized using Jurkat T-cell leukemia cells pulsed with 10 µM BrdU for 1.5 hours using an acid denaturation method.[7] We recommend using 5 µL of antibody per 1×10^6 cells in a 100 µL staining volume. Because conditions may vary, we recommend optimizing the amount of antibody used for each application. This product is supported for flow cytometry; for other uses, experimental conditions such as dilutions should be determined by individual investigators.

Caution

This product contains 5 mM sodium azide as a preservative. Sodium azide is an extremely toxic and dangerous compound, particularly when combined with acids or metals. Dispose of solutions containing sodium azide properly.

Preparing Antibodies

The unlabeled antibody and antibody conjugates are supplied in unit sizes of 500 µL as ready-to-use solutions in phosphate buffered saline (PBS), pH 7.2, containing 5 mM sodium azide. This is sufficient for 100 assays. These solutions are stable at 2–6°C for approximately twelve months.

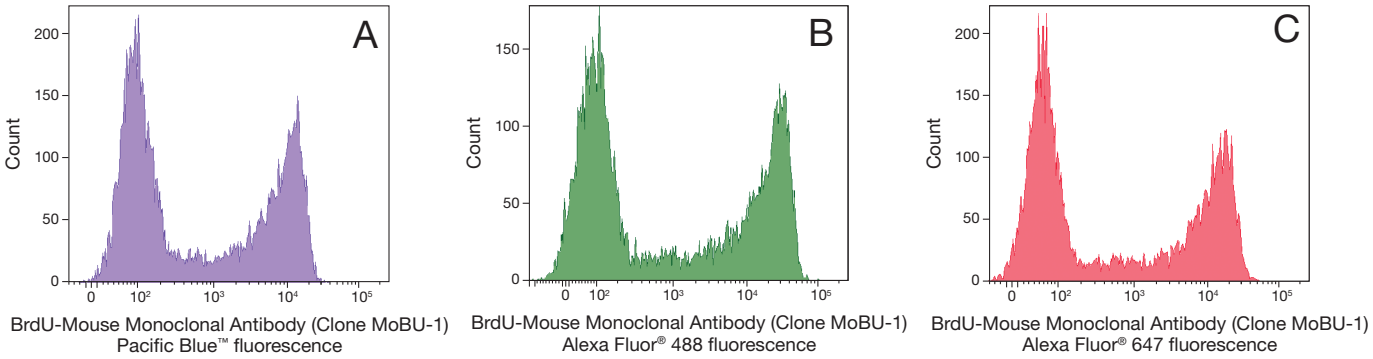

Figure 1. Jurkat T-cell leukemia cells were treated with 10 µM BrdU for 1.5 hours. The cells were then fixed in ethanol and stored overnight at ≤–20°C. An acid denaturation method[7] was used to prepare the cells before labeling them with BrdU-Mouse Monoclonal Antibody-Clone MoBU-1 to detect the incorporated BrdU. Plot A shows the Pacific Blue™ conjugate, plot B shows the Alexa Fluor® 488 conjugate, and plot C shows the Alexa Fluor® 647 conjugate, each using 5 µL antibody conjugate labeling 1×10^6 cells. Proliferating cells are clearly distinguished from non-proliferating cells.

References

1. Science 218, 474 (1982); **2.** Methods Cell Biol 41, 297 (1994); **3.** Cell Prolif 28, 571 (1995); **4.** PLoS Biology 5, 1120 (2007); **5.** Proc Natl Acad Sci USA 105, 2415 (2008); **6.** BioTechniques 44, 927 (2008); **7.** *Current Protocols in Cytometry vol 1*, JP Robinson, Ed., John Wiley & Sons Inc (2007).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
B35129	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Pacific Blue™	500 µL
B35139	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 488	500 µL
B35140	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 647	500 µL
B35141	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Unconjugated	500 µL
Related Products		
B23151	5-bromo-2′-deoxyuridine (BrdU)	100 mg
A10044	EdU (5-ethynyl-2′-deoxyuridine)	50 mg
A1034	Click-iT® EdU Pacific Blue™ Flow Cytometry Assay Kit	1 kit
C35002	Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Assay Kit	1 kit
A10202	Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit	1 kit
F10347	FxCycle™ Violet stain	1 set
F10348	FxCycle™ Far Red stain	1 set
S10274	SYTOX® AADvanced™ Dead Cell Stain Kit	500 assays
S10349	SYTOX® AADvanced™ Dead Cell Stain Kit	100 assays

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com.

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology
The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.