

BrdU-Mouse Monoclonal Antibodies (Clone MoBU-1) for Imaging Applications

Table 1. Contents and storage information.

Material	Conjugate	Concentration	Storage*
BrdU-Mouse Monoclonal Antibodies (Clone MoBU-1)	Unconjugated	0.1 mg/mL in phosphate buffered saline (PBS), pH 7.2, containing 5 mM sodium azide	• 2–6°C
	Biotin conjugate	0.2 mg/mL in phosphate buffered saline (PBS), pH 7.2, containing 5 mM sodium azide	
	Alexa Fluor® conjugate	0.2 mg/mL in phosphate buffered saline (PBS), pH 7.2, containing 5 mM sodium azide	• 2–6°C • Protect from light

*When stored as directed, the product is stable for 1 year.

Approximate fluorescence excitation/emission maxima: Alexa Fluor® 488 conjugate: 495/518 in nm; Alexa Fluor® 555 conjugate: 555/565 in nm; Alexa Fluor® 594 conjugate: 590/615 in nm; Alexa Fluor® 647 conjugate: 650/665 in nm.

Introduction

Cell proliferation can be measured with the thymidine analog BrdU (5-bromo-2'-deoxyuridine) following its incorporation into newly synthesized DNA and its subsequent detection with an anti-BrdU antibody. Although this method is rapidly being replaced with the recently developed click chemistry-based Click-iT® EdU assay,¹ an advantage of the antibody clone MoBU-1 is that it does not cross-react with the thymidine analog EdU (5-ethynyl-2'-deoxyuridine), thus making it valuable for use in dual pulse applications.

Traditionally, the dual pulse method, which is used for studying biological events such as neurogenesis and cell differentiation or evaluating anti-cancer drug efficacy, employs BrdU immunocytochemistry with iododeoxyuridine (IdU) or chlorodeoxyuridine (CldU), using multiple antibodies for detection. However, using sequential pulses of the thymidine analogs EdU and BrdU greatly simplifies dual pulse labeling, and allows a straightforward and reliable method of distinguishing BrdU- and EdU-labeled cells.

In addition to no cross-reactivity with EdU, the MoBU-1 clone has a high specificity for BrdU. This clone can also readily detect bromouridine (BrU) incorporated into RNA or BrdUTP incorporated into DNA strand breaks via the TUNEL assay. Samples labeled with the Alexa Fluor® conjugates of MoBU-1 can be directly imaged by fluorescence microscopy or high-content imaging. The unconjugated form and the biotin conjugate of this clone require a secondary antibody or a streptavidin conjugate for detection, respectively. The MoBU-1 clone is also available for flow cytometry applications; for ordering information on the flow cytometry BrdU antibodies, as well as the Click-iT® EdU assays for flow, imaging, and HCS, refer to the related product list (page 3).

Before Starting

Materials Required but Not Provided

- BrdU labeled cells or tissue
- Wash buffers (i.e., Phosphate Buffered Saline (PBS), pH 7.2–7.6)
- Fixative (i.e., 3.7% formaldehyde in PBS)
- Permeabilization reagent (i.e., 0.1% Triton® X-100 in PBS)
- DNA denaturation reagent (i.e., HCl or DNase)

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.

Experimental Protocols

Detection with BrdU antibodies requires not only fixed and permeabilized cells, but also denatured DNA for the BrdU antibody to gain access to the incorporated BrdU.^{2,3} The appropriate dilution of the antibody and the incubation time should be determined empirically. We recommend the following starting points:

- Unconjugated BrdU: 0.2–1 µg/mL (1:500–1:100 dilution)
- Biotin or Alexa Fluor® conjugate: 1–4 µg/mL (1:200–1:50 dilution)

Labeled samples are compatible with all slide preparation methods, including wet mount and prepared mounting media (i.e., *SlowFade*® Antifade and ProLong® Gold Kits). See Table 2 for the approximate fluorescence excitation/emission maxima for Alexa Fluor® dyes.

Table 2. Approximate fluorescence excitation/emission maxima.

Fluorophore	Excitation (nm)	Emission (nm)
Alexa Fluor® 488	495	519
Alexa Fluor® 555	555	565
Alexa Fluor® 594	590	615
Alexa Fluor® 647	650	670

References

1. Proc Natl Acad Sci USA 105, 2415 (2008); 2. Science 218, 474 (1982); 3. Methods Cell Biol 41, 297 (1994).

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

Cat. no.	Product Name	Unit Size
B35128	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - unconjugated *0.1 mg/mL*	350 µL
B35130	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 488 *0.2 mg/mL*	350 µL
B35131	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 555 *0.2 mg/mL*	350 µL
B35132	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 594 *0.2 mg/mL*	350 µL
B35133	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 647 *0.2 mg/mL*	350 µL
B35138	BrdU, Mouse Monoclonal Antibody (Clone MoBU-1) - biotin *0.2 mg/mL*	350 µL
Related Products		
B23151	5-bromo-2'-deoxyuridine (BrdU)	100 mg
B35129	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Pacific Blue™ *for flow cytometry* *100 tests*	1 each
B35139	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 488 *for flow cytometry* *100 tests*	1 each
B35140	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Alexa Fluor® 647 *for flow cytometry* *100 tests*	1 each
B35141	BrdU-Mouse Monoclonal Antibody (Clone MoBU-1) - Unconjugated *for flow cytometry* *100 tests*	1 each
C10337	Click-iT® EdU Alexa Fluor® 488 Imaging Kit *for 50 coverslips*	1 kit
C10338	Click-iT® EdU Alexa Fluor® 555 Imaging Kit *for 50 coverslips*	1 kit
C10339	Click-iT® EdU Alexa Fluor® 594 Imaging Kit *for 50 coverslips*	1 kit
C10340	Click-iT® EdU Alexa Fluor® 647 Imaging Kit *for 50 coverslips*	1 kit
C10350	Click-iT® EdU Alexa Fluor® 488 HCS Assay *2-plate size*	1 kit
C10351	Click-iT® EdU Alexa Fluor® 488 HCS Assay *10-plate size*	1 kit
C10352	Click-iT® EdU Alexa Fluor® 555 HCS Assay *2-plate size*	1 kit
C10353	Click-iT® EdU Alexa Fluor® 555 HCS Assay *10-plate size*	1 kit
C10354	Click-iT® EdU Alexa Fluor® 594 HCS Assay *2-plate size*	1 kit
C10355	Click-iT® EdU Alexa Fluor® 594 HCS Assay *10-plate size*	1 kit
C10356	Click-iT® EdU Alexa Fluor® 647 HCS Assay *2-plate size*	1 kit
C10357	Click-iT® EdU Alexa Fluor® 647 HCS Assay *10-plate size*	1 kit
A10034	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
A10044	EdU (5-ethynyl-2'-deoxyuridine)	50 mg
E10187	EdU (5-ethynyl-2'-deoxyuridine)	100 mg
P36930	ProLong® Gold Antifade Reagent	1 kit
S36936	SlowFade® Gold Antifade Reagent	1 mL
S36937	SlowFade® Gold Antifade Reagent *special packaging*	5 × 2 mL

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