EdU (5-ethynyl-2'-deoxyuridine)

Catalog nos. A10044, E10187, E10415

Table 1. Contents and storage information.

Material	Amount	Storage*	Stability
EdU (5-ethynyl-2'-deoxyuridine)	50 mg (A10044) 500 mg (E10187) 5 g (E10415)	• ≤–20˚C • Desiccate	When stored as directed, the product is stable for 1–2 years

Introduction

EdU (5-ethynyl-2'-deoxyuridine) is a novel alternative for BrdU (5-bromo-2'-deoxyuridine) assay to directly measure active DNA synthesis or S-phase synthesis of the cell cycle. EdU is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis.[1] Detection of EdU is based on a click reaction,[1-5] which is a copper (I) catalyzed reaction between an azide and an alkyne. The EdU contains the alkyne which can be reacted with the an azide-containing detection reagent, to form a stable, triazole ring (Figure 1).

The advantages of the click reaction with EdU labeling are readily evident while performing the assay. The small size of the detection azide allows the use of mild conditions to access EdU incorporated into the DNA. This is in contrast to BrdU-based assays that require DNA denaturation (typically using HCl, heat, or digestion with DNase) to expose the BrdU for detection with an anti-BrdU antibody (Figure 2). Eliminating the denaturation step allows for a simple, fast protocol producing more reproducible results and measurements which are easily multiplexed with relevant antibody based targets including phospho-histone H3, Ki-67, and cyclin B1 by flow cytometry, fluorescence microscopy, or high-throughput imaging (HCS) (Figures 3 and 4).

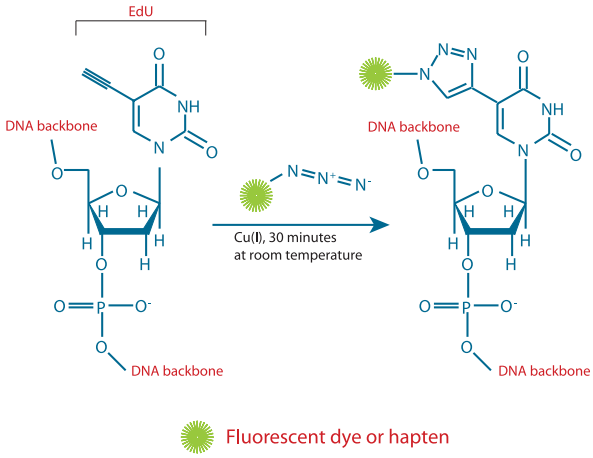

Figure 1. Click reaction between EdU and azide-modified dye or hapten.

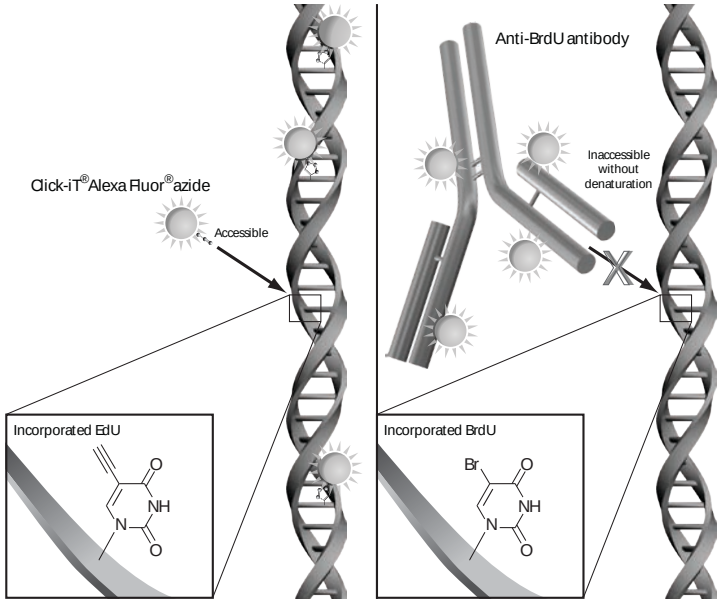

Figure 2. Detection of the incorporated EdU with Alexa Fluor® azide versus incorporated BrdU with an anti-BrdU antibody. The small size of the Alexa Fluor® azide eliminates the need to denature the DNA.

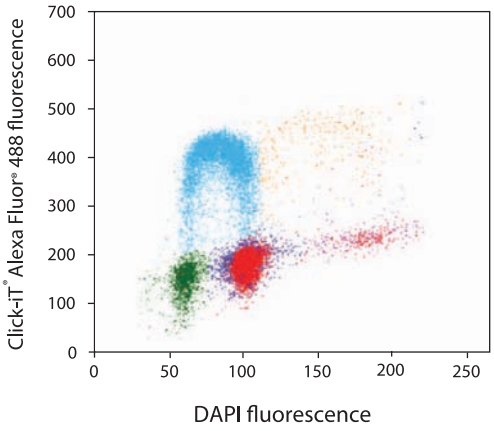

Figure 3. Multiparameter analyses with EdU by flow cytometry. After treating U266 myeloma cells with 10 nM nocodazole for 15 hours, the cells were incubated with 10 µM EdU for 1 hour. Cells were harvested and dead cells were labeled with the LIVE/DEAD® Fixable Near-IR Dead Cell Stain (Invitrogen Cat. no. L10119) prior to fixation with 4% paraformaldehyde in PBS. Cells were saponin-permeabilized and EdU was detected with the Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Kit (Invitrogen Cat. no. C35002). Phosphorylated histone H3 and cyclin B1 were labeled using Alexa Fluor® 647 rat anti-histone H3 (pS28) and purified mouse anti-Cyclin B1 (GSN-1) complexed with Zenon® R-Phycoerythrin Mouse IgG_1 Labeling reagent (Invitrogen Cat. no. Z25055), respectively. DNA content was labeled with DAPI (Invitrogen Cat. no. D1306). Acquisition and analysis was performed on a BD™ LSRII flow cytometer using 355 nm, 488 nm, and 633 nm excitations in 5-color analysis with BD. EdU signal was plotted against DNA content and shows an increase of cells in G2M phase with the nocodazole treatment.

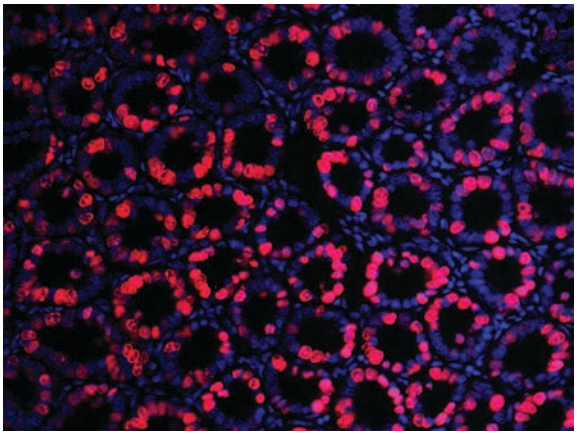

Figure 4. Proliferating cells in rat ileum. Rats were treated with a 2 hour pulse of EdU administered I.P. (160 µg/g body weight). A 5 µm thick paraffin embedded tissue was deparaffinized with standard xylene based protocol. Proliferating cells were detected with the Click-iT® EdU Alexa Fluor® 594 Imaging Kit (Cat. no. C10339). Nuclei were stained with the blue-fluorescent counterstain Hoechst 33342 (Cat. no. H1399).

Before You Begin

Preparing Stock Solution

EdU is readily soluble in DMSO, alcohol, water, or aqueous buffers. For use with *in vitro* applications, prepare 10 mM EdU stock solution in DMSO or aqueous buffer. Store 10 mM EdU stock solution at ≤–20˚C for one year.

EdU has a characteristic 288 nm absorption peak which can be used to accurately quantitate stock solutions by absorbance using the extinction coefficient of 12,000 $cm^{-1}M^{-1}$ in methanol. A 10 mg/mL solution (39.6 mM) when diluted 1/1,000 in methanol gives an absorbance of 0.475 at 288 nm.

Handling and Disposal

EdU is a nucleoside analog which can be incorporated into DNA. Handle and dispose of EdU in compliance with all pertaining local regulations. When EdU is dissolved in DMSO, which is known to facilitate the entry of organic molecules into tissue, use additional precautions appropriate for the hazards posed by such materials.

EdU Toxicity

Pharmacotoxicity data for EdU is not known. Potential toxicity effects can be reduced by reducing the amount of EdU. Studies of tumorigenicity and cytotoxicity in Zebra Finch and *Taeniopygia guttata*, showed no abnormal results in a variety of tissues and appeared to be well tolerated. In these studies, the dosage regiment was the same as used for BrdU labeling.

Experimental Protocols

EdU Labeling

In initial experiments, we recommend testing a range of EdU concentrations to determine the optimal concentration. If currently using a BrdU-based assay, a similar concentration and duration to BrdU is a good starting concentration for EdU. Lower amounts of EdU can be used to achieve equivalent brightness of labeling as BrdU. The optimal concentration may vary depending upon the duration of the pulse, with lower concentrations recommended for longer incubations. General recommendations are listed below. Details on using EdU, including references, cell images, and data are available at www.invitrogen.com/edu

- Cultured cells—Acceptable EdU incorporation has been observed with cultured cells including mammalian and plant labeled with 0.1–10 µM EdU for 0.5–3 hours.
- Whole animal—Acceptable EdU incorporation has been observed following injection or media incubation (Table 2).

Table 2. Using EdU in animal species.

Species	Reference*
Nematode (*C. elegans*)	Dorsett M, Westlund B, Schedl T (2009) Genetics 183: 233–247
Flatworm (marine)	BioProbes 61
Cricket	Bando T, Mito T, Maeda Y et al. (2009) Development 136: 2235–2245
Mouse	Salic, A (2008) Proc Natl Acad Sci USA 105: 2415–2420 Bonaguidi MA, Peng CY, McGuire T et al. (2008) J Neurosci 28: 9194–9204 Kharas MG, Janes MR, Scarfone VM et al. (2008) J Clin Ivest 118: 3038–3050 Zeng C et al. (2010) Brain Res 1319: 21–32
Rat	Scientific poster, ASCB 2007
Zebrafish larva	BioProbes 57
Zebra finch	Scientific poster ASCB 2007
Human-derived stem cells	McCord AM, Jamal M. Williams ES et al. (2009) Clin Cancer Res 15: 5145–5153 Momcilovic O, Choi S, Varum S et al. (2009) Stem Cells 27: 1822–1835

*Visit www.invitrogen.com/edu for links to PubMed entries, scientific poster, or detailed protocol.

Dual Pulse Labeling

Follow these guidelines to perform dual labeling of cultured cells and tissue by combining EdU with BrdU labeling (Figure 5):

- Use EdU for the first pulse and BrdU for the second pulse.
- Removal of EdU from the media is not required in cultured cells when BrdU is added as the second label.
- Addition of BrdU to culture media containing EdU results in preferential incorporation of BrdU into the DNA with the exclusion of EdU, while simultaneous addition of EdU with equimolar or half equimolar BrdU to the media results in only BrdU incorporation. This simplifies the dual labeling protocol by eliminating the wash step normally required to remove the first label from the culture media prior to addition of the second label.
- Process cells or tissues after dual pulse labeling using a proven BrdU protocol. Combine the click detection protocol with the BrdU protocol.
- For cultured cells, the BrdU protocol usually requires an alcohol fixation followed by some method of DNA denaturation. After the DNA denaturation/neutralization step in the BrdU protocol, cells are first click labeled for the detection of EdU followed by an antibody labeling protocol for the detection of BrdU.
- Select a BrdU antibody which does not have cross-reactivity to EdU. Many BrdU antibodies have been shown to have some amount of cross-reactivity with incorporated EdU.

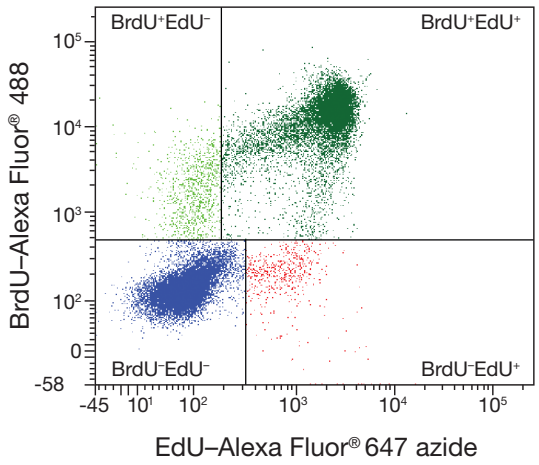

Figure 5. Dual pulse labeling with EdU and BrdU. TF-1 erythroblast cells were pulsed with 20 µM EdU for 1 hour followed by 10 µM BrdU for 1 hour. The cells were fixed in ethanol, and an acid denaturation method was used before labeling with anti-BrdU (Clone MoBU-1)-Alexa Fluor® 488 conjugate (Cat. no. B35139) and Click-iT® EdU-Alexa Fluor® 647 azide (Cat. no. A10202). Data were collected with a BD™ LSR II flow cytometer using 488 nm excitation with a 530/30 bandpass filter, and 633 nm excitation with a 660/20 bandpass filter. Cells colored blue are negative for both EdU and BrdU (lower left quadrant); cells colored dark green are positive for both EdU and BrdU (upper right quadrant); cells colored red are positive for EdU but negative for BrdU (lower right quadrant); cells colored light green are negative for EdU but positive for BrdU (upper right quadrant).

EdU detection

Incorporated EdU can be detected with a Click-iT® EdU Kit (Table 3) or with an available dye- or hapten-containing azide (Table 4). Two of the fluorescent dyes, Alexa Fluor® 488 and Oregon Green® 488 can be used as fluorescent dyes or haptens with an anti-dye antibody.

Table 3. Click-iT® EdU Kits for flow cytometry and microscopy.

Instrument platform	Number of samples	Available fluorophores	Cat. no.	Notes
Flow cytometry	50 assays based upon a 0.5 mL volume	Alexa Fluor® 488 dye	C35002	• Includes 2 cell-cycle dyes compatible with the detection fluorophore • Not interchangeable with imaging assays
		Alexa Fluor® 647 dye	A10202	
		Pacific Blue™ dye	A10034	
Fluorescence microscopy (Imaging)	50 coverslips	Alexa Fluor® 488 dye	C10337	• Includes blue-fluorescent nuclear counterstain, Hoechst 33342 • Not interchangeable with flow cytometry assays
		Alexa Fluor® 555 dye	C10338	
		Alexa Fluor® 594 dye	C10339	
		Alexa Fluor® 647 dye	C10340	

Table 4. Dye- and hapten-containing azides.

Azide	Use	Cat. no.	Excitation*	Emission*
Alexa Fluor® 488 dye	Fluorescent dye or hapten	A10266	495	519
Alexa Fluor® 594 dye	Fluorescent dye	A10270	590	617
Alexa Fluor® 647 dye	Fluorescent dye	A10277	650	665
Oregon Green® 488 dye	Fluorescent dye or hapten	O10180	496	524

*Excitation and emission maxima in nm.

References

1. Proc Natl Acad Sci U S A 105, 2415 (2008); **2.** ChemBioChem 4, 1147 (2003); **3.** J Am Chem Soc 125, 3192 (2003); **4.** Angew Chem Int Ed Engl 41, 2596 (2002); **5.** Angew Chem Int Ed Engl 40, 2004 (2001).

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

Cat. no.	Product Name	Unit Size
A10044	EdU (5-ethynyl-2′-deoxyuridine)	50 mg
E10187	EdU (5-ethynyl-2′-deoxyuridine)	500 mg
E10415	EdU (5-ethynyl-2′-deoxyuridine)	5 g
Related Products		
C10337	Click-iT® EdU Alexa Fluor® 488 Imaging Kit *50 coverslips*	1 kit
C10338	Click-iT® EdU Alexa Fluor® 555 Imaging Kit *50 coverslips*	1 kit
C10339	Click-iT® EdU Alexa Fluor® 594 Imaging Kit *50 coverslips*	1 kit
C10340	Click-iT® EdU Alexa Fluor® 647 Imaging Kit *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
C10356	Click-iT® EdU Alexa Fluor® 647 HCS Assay *2-plate size*	1 kit
C10355	Click-iT® EdU Alexa Fluor® 594 HCS Assay *10-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 *50 assays*	1 kit
C35002	Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Assay Kit *50 assays*	1 kit
A10202	Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit *50 assays*	1 kit
A10266	Alexa Fluor® 488 azide (Alexa Fluor® 488 5-carboxamido-(6-azidohexanyl), bis(triethylammonium salt))	0.5 mg
A10270	Alexa Fluor® 594 azide (Alexa Fluor® 594 carboxamido-(6-azidohexanyl), bis(triethylammonium salt))	0.5 mg
A10277	Alexa Fluor® 647 azide, tris(triethylammonium salt)	0.5 mg
O10180	Oregon Green® 488 azide (Oregon Green® 488 6-carboxamido-(6-azidohexanyl), triethylammonium salt)	0.5 mg
A11094	anti-Alexa Fluor® 488, rabbit IgG fraction *1 mg/mL*	0.5 mL
A889	anti-fluorescein/Oregon Green®, rabbit IgG fraction *1 mg/mL*	0.5 mL
A982	anti-fluorescein/Oregon Green®, rabbit IgG fraction, biotin-XX conjugate *1 mg/mL*	0.5 mL
A6413	anti-fluorescein/Oregon Green®, rabbit IgG Fab fragment *0.5 mg/mL*	0.5 mL
A6421	anti-fluorescein/Oregon Green®, mouse IgG_{2a}, monoclonal 4-4-20	0.5 mg
Q10137MP	Qdot® 565 goat anti-fluorescein conjugate *2 µM* *whole IgG*	0.5 mL
A11090	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A11091	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 594 conjugate *1 mg/mL*	0.5 mL
A11095	anti-fluorescein/Oregon Green®, goat IgG fraction *1 mg/mL*	0.5 mL
A11096	anti-fluorescein/Oregon Green®, goat IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
Q15421MP	Qdot® 655 goat anti-fluorescein conjugate *2 µM* *whole IgG*	200 µL
Q15421MP	Qdot® 565 goat anti-fluorescein conjugate *2 µM* *whole IgG*	200 µL
A21250	anti-fluorescein/Oregon Green®, rabbit IgG fraction, R-phycoerythrin conjugate *2 mg/mL*	250 µL
A21251	anti-fluorescein/Oregon Green®, rabbit IgG fraction, horseradish peroxidase conjugate	0.5 mg
B35128	BrdU, mouse monoclonal antibody (Clone MoBU-1) - unconjugated *0.1 mg/mL*	350 µL
B35129	BrdU, mouse monoclonal antibody (Clone MoBU-1) - Pacific Blue™ *for flow cytometry* *100 tests*	1 each
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 *0.1 mg/mL*	350 µL
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

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