Breakthrough cell proliferation assays

Click-iT® EdU cell proliferation assays replace BrdU

- → Accurate, consistent performance—no denaturation steps or harsh treatments required
- → Simple method—works the first time, every time, in less time
- → Content-rich results—better preservation of cell morphology, antigens, and dsDNA integrity

Detection of cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anticancer drugs. The most accurate method utilizes direct measurement of new DNA synthesis. Traditionally, this is performed by incorporating the nucleoside analog bromodeoxyuridine (BrdU) into DNA, followed by detection with an anti-BrdU antibody. Although effective, this method requires DNA denaturation (using HCl, heat, or DNase) to expose the BrdU to the antibody—a step that can be lengthy and difficult to perform consistently, and that can adversely affect the sample. The Click-iT® EdU cell proliferation assays eliminate the need to denature DNA, providing a superior alternative to the standard BrdU antibody-based method for measuring cell proliferation by flow cytometry or high-throughput imaging (Table 1, Figures 1–3).

Better than BrdU

The Click-iT® advantage is in the chemistry—small, unique bioorthogonal labeling and detection moieties that react very efficiently and specifically with one another. EdU (5-ethynyl-2´-deoxyuridine) is a nucleoside analog containing an alkyne. In a copper-catalyzed reaction, the alkyne reacts with an azide, forming a very stable covalent bond—unlike the noncovalent bond between an antibody and an antigen. The small size of the Alexa Fluor® azide allows efficient access to the DNA without the need for harsh sample treatment, simplifying the assay considerably yet generating the same results.

Table 1. Click-iT® chemistry provides a superior method.

	Click-iT® EdU kits	BrdU detection
Harsh DNA denaturation	**No**	Yes
Easy to perform	**Yes**	No
Mild fixation and permeabilization	**Yes**	No

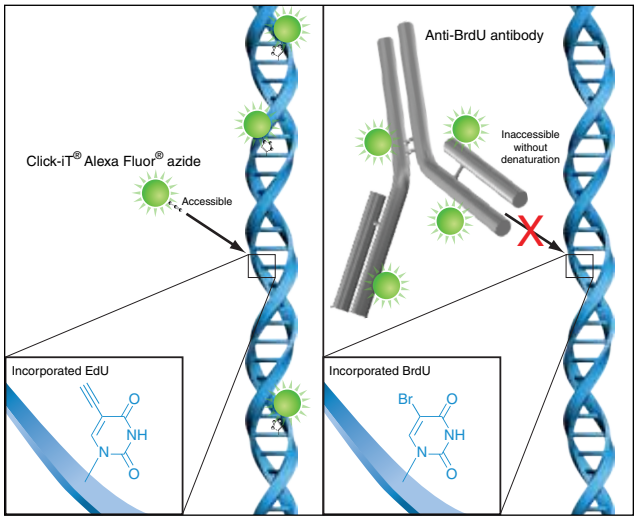

Figure 1. Detection of incorporated EdU with the Alexa Fluor® 488 azide versus incorporated BrdU with an anti-BrdU antibody. The small size of the Alexa Fluor® azide eliminates the need to denature DNA in order for the detection reagent to gain access to the modified base.

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Everything you need, easy to perform

The Click-iT® EdU protocol is based on aldehyde fixation and detergent permeabilization steps for immunohistochemical antibody labeling, but EdU is compatible with other fixation/permeabilization agents including saponin and methanol. In just three steps you'll be ready to analyze your cell proliferation data:

1. Treat cells with EdU
2. Fix and permeabilize cells
3. Detect S-phase cells with Click-iT® detection cocktail for 30 min, wash once, then analyze

The Click-iT® EdU cell proliferation assay kits provide everything you need to get started. The EdU assay kits can also be multiplexed with antibodies to detect surface and intracellular biomarkers. Qdot® nanocrystals, R-PE, and R-PE tandems should be used after the Click-iT® detection reaction for optimal results. GFP antibodies should be used to achieve fluorescence and detect GFP. Antibodies labeled with APC and small organic dyes such as Alexa Fluor® 647 dye are completely compatible with the Click-iT® detection reaction. With Click-iT® EdU, you have a truly simple, accurate, multiplex-compatible, and reliable assay for cell proliferation.

Ordering information

Product	Quantity	Cat. No.
Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Assay Kit	1 kit, 50 assays	C35002
Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit	1 kit, 50 assays	A10202
Click-iT® EdU Pacific Blue™ Flow Cytometry Assay Kit	1 kit, 50 assays	A10034
Click-iT® EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay	1 kit, 2 plates 1 kit, 10 plates	C10350 C10351
Click-iT® EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay	1 kit, 2 plates 1 kit, 10 plates	C10354 C10355
Click-iT® EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay	1 kit, 2 plates 1 kit, 10 plates	C10356 C10357
Click-iT® EdU Alexa Fluor® 488 Imaging Kit	1 kit, 50 coverslips	C10337
Click-iT® EdU Alexa Fluor® 594 Imaging Kit	1 kit, 50 coverslips	C10339
Click-iT® EdU Alexa Fluor® 647 Imaging Kit	1 kit, 50 coverslips	C10340
Click-iT® EdU Microplate Assay	400 assays, 1 kit	C10214
EdU (5-ethynyl-2′-deoxyuridine)	50 mg	A10044
EdU (5-ethynyl-2′-deoxyuridine)	500 mg	E10187
For current prices, please visit www.invitrogen.com.		

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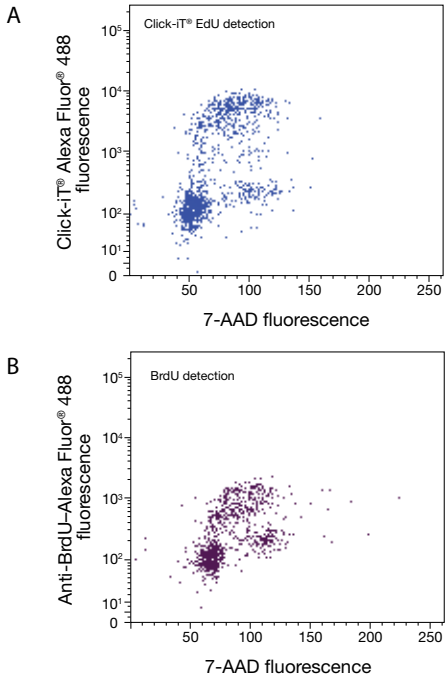

Figure 2. More precise detection obtained using the Click-iT® EdU reagents compared to BrdU assay. (A) Results obtained using the new Click-iT® EdU detection method, showing a dual-parameter plot of Click-iT® Alexa Fluor® 488 azide vs. 7-AAD cell cycle staining. (B) Results obtained using a standard acid denaturation method, showing a dual-parameter plot of anti-BrdU–Alexa Fluor® 488 vs. 7-AAD cell cycle staining.

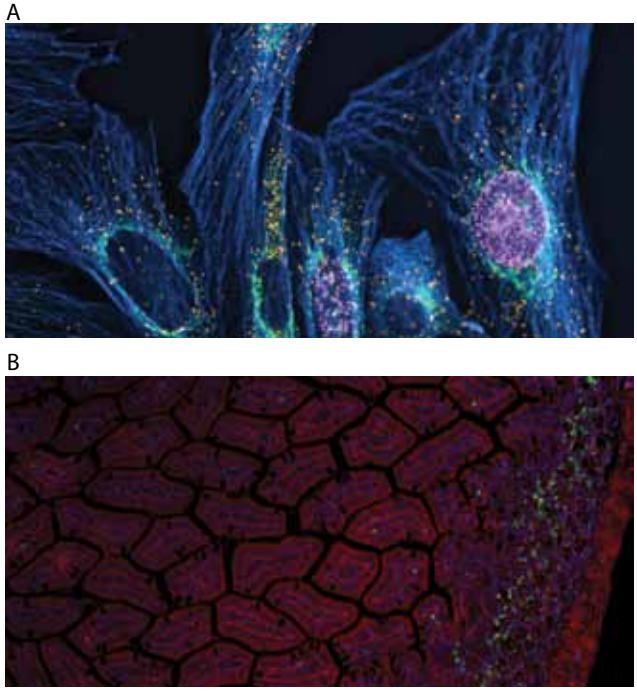

Figure 3. Click-iT® EdU enables content-rich results. Newly synthesized DNA detected with Click-iT® EdU in (A) cells and (B) tissue.

Molecular Probes® offers fluorescence technology that enables uniquely powerful labeling and detection solutions for cellular analysis.

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