

# 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).

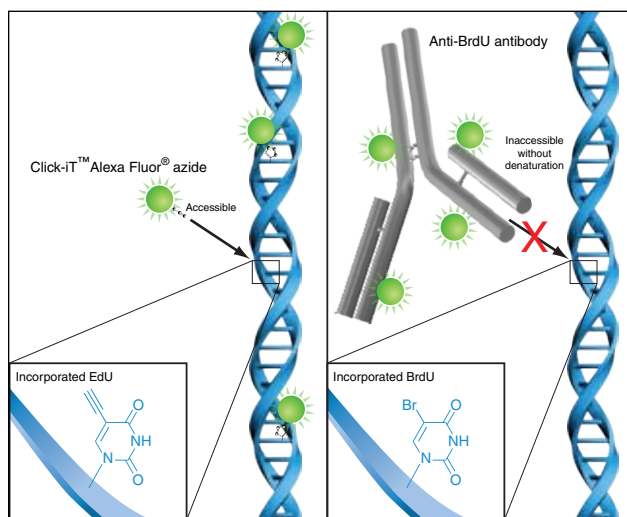
### Better than BrdU

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® 488 azide allows efficient access to the DNA without the need for harsh sample treatment, thus 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 the incorporated EdU with the Alexa Fluor® 488 azide versus incorporated BrdU with an anti-BrdU antibody.** The small size of the Alexa Fluor® 488 azide eliminates the need to denature DNA in order for the detection reagent to gain access to the modified base.

## 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

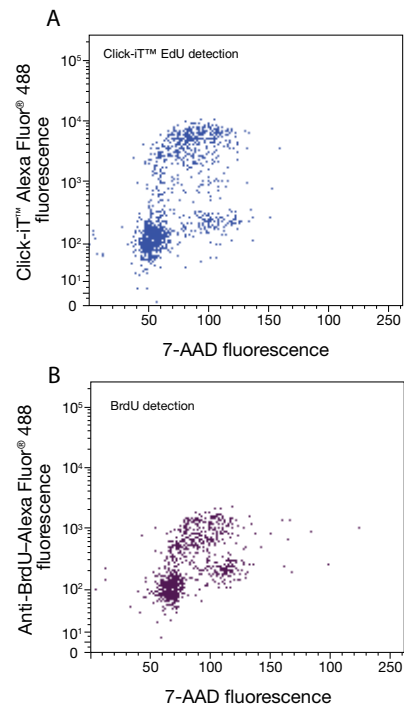
The Click-iT™ EdU cell proliferation assay kits provide everything you need to get started. The EdU assay kits can also be multiplexed with antibodies to detect surface and intracellular biomarkers. Qdot® nanocrystals, R-PE, and R-PE tandems should be used after the Click-iT™ detection reaction for optimal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

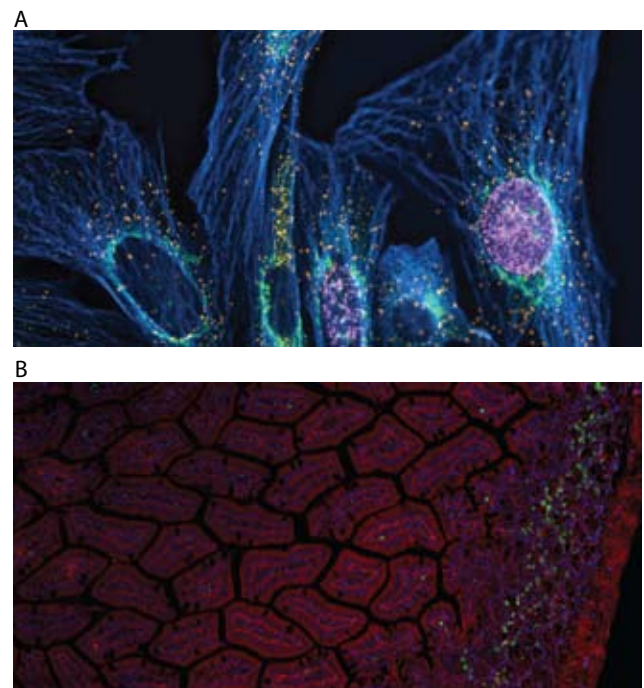
| Product  | Quantity             | Cat. no. |
|--|----------------------|----------|
| Click-iT™ EdU Alexa Fluor® 488 Flow Cytometry Assay Kit            | 1 kit, 50 assays     | C35002   |
| Click-iT™ EdU Alexa Fluor® 647 Flow Cytometry Assay Kit            | 1 kit, 50 assays     | A10202   |
| Click-iT™ 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, 10 plates     | A10028   |
| Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay | 1 kit, 10 plates     | C10082   |
| Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay | 1 kit, 10 plates     | C10081   |
| Click-iT™ EdU Alexa Fluor® 488 Imaging Kit                         | 1 kit, 50 coverslips | C10083   |
| Click-iT™ EdU Alexa Fluor® 594 Imaging Kit                         | 1 kit, 50 coverslips | C10084   |
| Click-iT™ EdU Alexa Fluor® 647 Imaging Kit                         | 1 kit, 50 coverslips | C10085   |
| EdU (5-ethynyl-2'-deoxyuridine)                                    | 50 mg                | A10044   |

For current prices, please visit [www.invitrogen.com](http://www.invitrogen.com).

Visit [www.invitrogen.com/edu](http://www.invitrogen.com/edu) for a free EdU-cation.



**Figure 2—Results obtained using the Click-iT™ EdU reagents typically surpass BrdU assay results.** 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 with Click-iT™ EdU in (A) cells and (B) tissue.