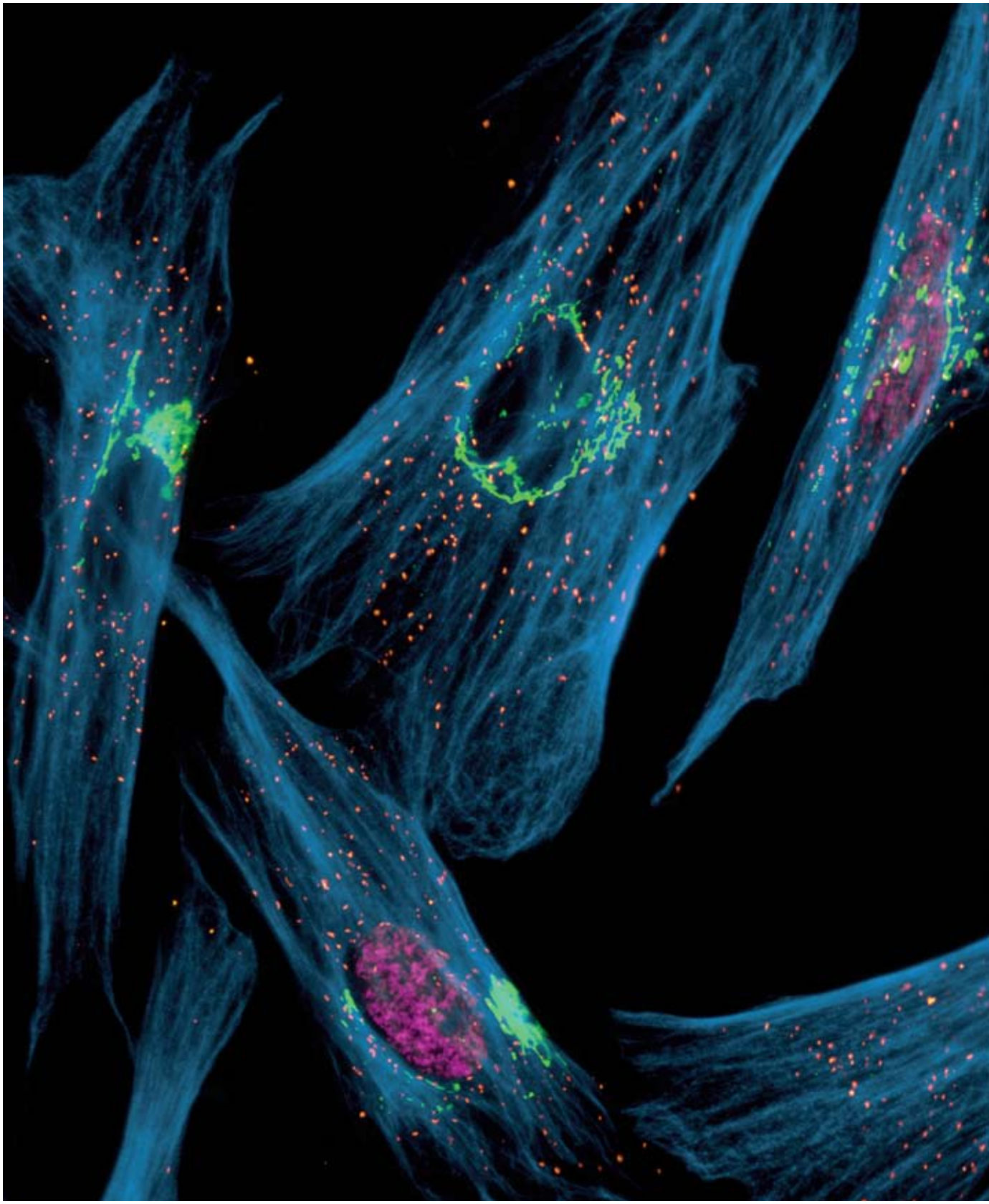

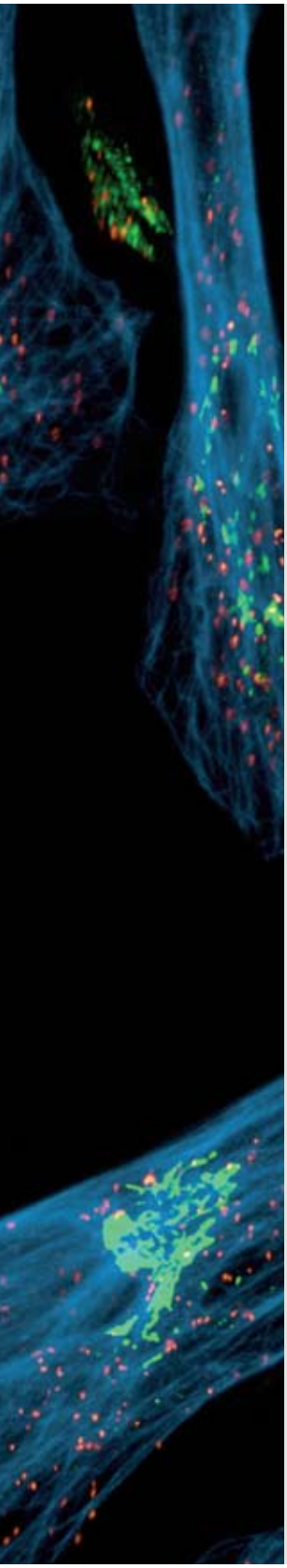

Click chemistry: Making biodiscovery a snap

REPLACE CUMBERSOME BrdU ASSAYS WITH THE SIMPLICITY OF EdU.

Whether your preferred cellular detection method is immunofluorescence or direct fluorescent labeling, what you really need are all of the advantages and none of the drawbacks. Instead, researchers often find themselves trading one set of challenges for another. The traditional detection method employs antibodies, which provide highly specific labeling; however, for an antibody to gain access to the target antigen, long incubations or harsh treatments are often required. Direct fluorescent detection, another common method, offers flexibility and ease of use. But when the label is larger than the molecule you want to visualize, the function of that molecule can be compromised. "Click" chemistry offers the best of both worlds. This unique technology provides a means of direct fluorescent labeling and detection without the background that compromises conventional amine- and thiol-reactive chemistries. Click-iT™ EdU assays offer all of the advantages of this powerful technology, providing a fast, easy, and accurate method for detecting cell proliferation (Figure 1).

Figure 1—Multicolor imaging is a snap with Click-iT™ EdU. Muntjac cells were treated with 10 µM EdU for 45 minutes. Cells were then fixed and permeabilized, and EdU that had been incorporated into newly synthesized DNA was detected using the far red–fluorescent Click-iT™ EdU Alexa Fluor® 647 Imaging Kit (Cat no. C10085). Tubulin was labeled with a mouse anti-tubulin antibody and visualized with an Alexa Fluor® 350 goat anti–mouse IgG antibody (Cat. no. A11045). The Golgi complex was stained with the green-fluorescent Alexa Fluor® 488 conjugate of lectin HPA from *Helix pomatia* (edible snail) (Cat. no. L11271), and peroxisomes were labeled with a rabbit anti-peroxisome antibody and visualized with an orange-fluorescent Alexa Fluor® 555 donkey anti–rabbit IgG antibody (Cat. no. A31572).

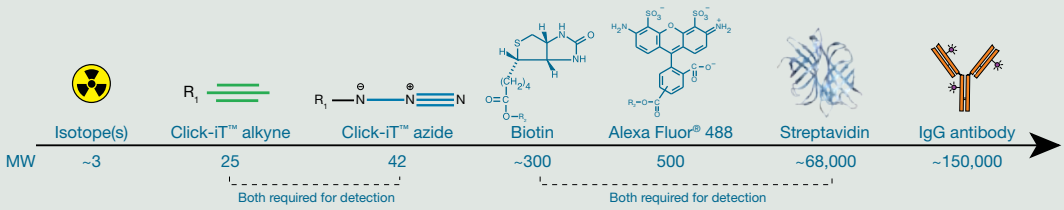

Figure 2—Relative size of detection molecules commonly used in cellular analysis. Because the azide and alkyne moieties can be used interchangeably to optimize labeling configurations, R_1 can be either the biomolecule of interest or the detection reagent. For biotin and the fluorophore, R_2 is the biomolecule of interest.

Click chemistry describes a class of chemical reactions that use bio-orthogonal or biologically unique moieties to label and eventually detect a molecule of interest using a two-step procedure. Click reactions have several characteristics: the reaction between the detection moieties is efficient; no extreme temperatures or solvents are required; the reaction product is stable; the components of the reaction are bioinert; and perhaps most importantly, no side reactions occur—the label and detection tags react selectively and specifically with one another.[1–4] This final point is the greatest advantage of this powerful detection technique; click chemistry–labeled molecules can be applied to complex biological samples and detected with unprecedented sensitivity, thanks to extremely low background.

Small size translates to big advantages

The Click-iT™ EdU cell proliferation assays are based on a two-step click reaction involving the copper-catalyzed triazole formation of an azide and an alkyne. Both azides and alkynes are biologically unique, inert, stable—and extremely small (Figure 2). These moieties can be used interchangeably; either one can be used to tag the molecule of interest while the other is used for subsequent detection. The label is small enough that tagged molecules (e.g., nucleotides, sugars, and amino acids) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers.

Click chemistry fills the void when methods such as direct labeling or the use of antibodies fail or fall short. For example, depending on the size of the molecule of interest, a direct bioconjugate with biotin or a fluorophore may adversely affect the biological function of that molecule. And antibodies and other indirect detection methods may be too large to access the antigen of interest in tissues or cells without resorting to harsh permeabilization or long incubations. With its small "footprint", the Click-iT™ detection molecule can easily penetrate complex samples, including intact (supercoiled) DNA, with only mild permeabilization required.

Click-iT™ EdU—fast, easy, and accurate detection of cell proliferation

Click-iT™ EdU cell proliferation assays are top of the class for direct and accurate detection of new DNA synthesis. These assays not only measure proliferation of individual cells, but also can detect proliferating cells on virtually any platform (Table 1). Click-iT™ assays use a modified nucleoside, EdU (5-ethynyl-2´-deoxyuridine), that is incorporated during DNA synthesis. Unlike assays using bromodeoxyuridine (BrdU), Click-iT™ EdU assays are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside (Figure 3).

Table 1—Click-iT™ EdU assays.

Platform	Amount of EdU included	Number of samples	Available fluorophores *	Notes
Flow cytometry	10 mg	50 assays based upon a 0.5 ml volume	Alexa Fluor® 488 Alexa Fluor® 647 Pacific Blue™ dye	• Includes two cell cycle dyes compatible with detection fluorophore • Not interchangeable with imaging assays
High-throughput imaging (HCS)	0.255 mg (2-plate) 1.28 mg (10-plate)	2 × 96 tests (2-plate) 10 × 96 tests (10-plate)	Alexa Fluor® 488 Alexa Fluor® 594 Alexa Fluor® 647	• Includes Hoechst 33342 for cell registration or cell cycle analyses • Not interchangeable with flow cytometry assay
Fluorescence microscopy	5 mg	50 coverslips	Alexa Fluor® 488 Alexa Fluor® 594 Alexa Fluor® 647	• Includes blue-fluorescent nuclear counterstain, Hoechst 33342 • Not interchangeable with flow cytometry assay

* Each kit contains one fluorophore. See product list for kit catalog numbers.

For BrdU detection, DNA is denatured with DNase, heat, or HCl; these treatments can destroy antigen recognition sites or make it difficult to perform simultaneous cell cycle analyses on the same sample, as many dyes for cell cycle analysis require dsDNA (Table 2, Figure 4). Click-iT™ assays avoid the harsh treatments required by BrdU assays, providing a method that is more reliable and easier to perform. And although BrdU assays performed in tissue samples involve long incubations—from several hours to overnight—Click-iT™ EdU detection reactions are complete in less than 80 minutes, and often in under 30 minutes.

Cell walls are no barrier for Click-iT™ EdU

Cell walls typically provide a barrier to detecting cell proliferation in plant cells. Using antibodies not only requires DNA denaturation, but also requires that the cell wall is digested. However, cell wall–digesting enzymes often contain impurities that can decrease the reliability of the assay. Furthermore, the additional steps required can make BrdU assays labor intensive and time consuming. But even in plant cells, Click-iT™ EdU assays involve only a mild fixation and permeabilization step—no DNA denaturation or cell wall digestion is required (Figure 5). Click-iT™ EdU assays are faster, more accurate, and extremely sensitive and reliable, even on tough samples.

Multiplexed analyses are a snap with Click-iT™ EdU

Click-iT™ EdU assays are ideal for multiplexed analysis in both cells and tissues. Because only a very mild fixation and permeabilization step is required for entry of the detection moiety, not only is dsDNA retained, but antigen recognition sites are preserved. Although we recommend starting with an amount of EdU equal to the amount of BrdU normally used, researchers can often use less EdU or reduce the incubation time and still obtain the same or even better signal than that provided by the BrdU assay. After EdU is incorporated, simply fix and permeabilize, then perform the Click-iT™ detection →

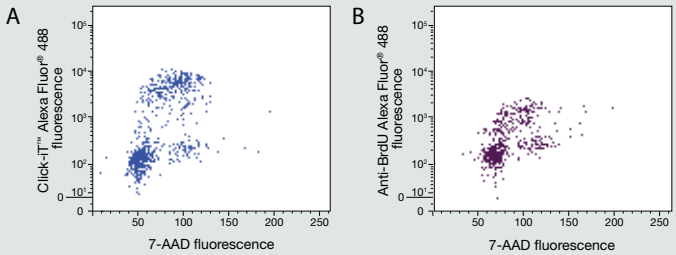

Figure 4—Results obtained using the Click-iT™ EdU reagents typically surpass those from the BrdU assay. (A) Results obtained using the new Click-iT™ EdU detection method, showing a dual-parameter plot of Click-iT™ Alexa Fluor® 488 azide vs. 7-AAD cell cycle staining. **(B)** Results using the standard acid denaturation method for the antibody-based detection of incorporated BrdU, showing a dual-parameter plot of anti-BrdU Alexa Fluor® 488 vs. 7-AAD cell cycle staining.

Table 2—Properties of several cell cycle stains compatible with Click-iT™ EdU.

Dye	Characteristics
DAPI	AT-selective
Hoechst 33342	AT-selective
Propidium iodide	Intercalator; binds both DNA & RNA; RNase recommended
7-AAD	GC-selective

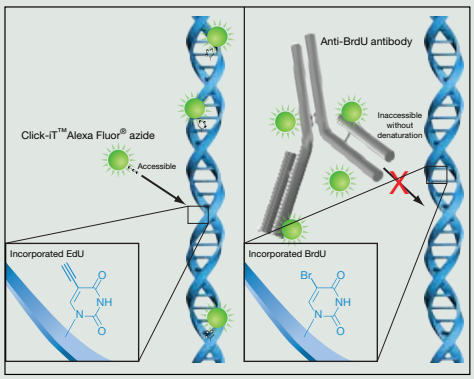

Figure 3—Detection of the incorporated EdU with the Alexa Fluor® azide versus incorporated BrdU with an anti-BrdU antibody. The small size of the Alexa Fluor® azide eliminates the need to denature the DNA in order for the detection reagent to gain access to the nucleoside.

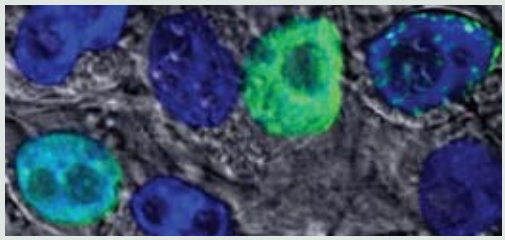

Figure 5—Cell wall digestion is not required with Click-iT™ EdU. *Medicago sativa* (alfalfa) suspension cultures were incubated with 10 µM EdU for 3 hours. Cells were then fixed and permeabilized. EdU that had been incorporated into newly synthesized DNA was detected with the Click-iT™ EdU Alexa Fluor® 488 Imaging Kit (green fluorescence, Cat. no. C10083). Nuclei were stained with blue-fluorescent DAPI. Six confocal sections were overlaid onto a differential interference contrast image. Image contributed by Ferhan Ayaydin, Cellular Imaging Laboratory, Biological Research Center, Szeged, Hungary.

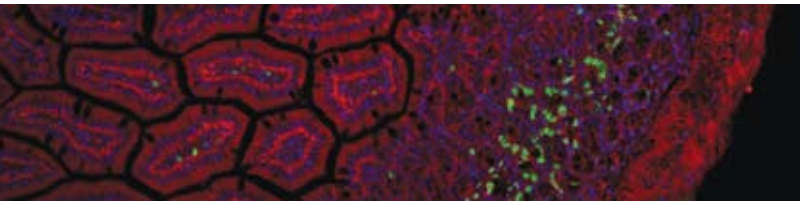

Figure 6—Proliferating cells labeled *in vivo* with the Click-iT™ EdU assay. EdU from the Click-iT™ EdU Alexa Fluor® 488 Imaging Kit (Cat. no. C10083) was administered to mice intraperitoneally 2 hours before sacrifice. After intestinal tissues were formalin fixed and paraffin embedded, EdU was labeled with the Click-iT™ reaction, using approximately 250 µl of reaction cocktail per slide. The tissue sections were then washed and treated with mounting medium containing DAPI. EdU-positive cells are labeled green, nuclei are stained blue, and red autofluorescence was enhanced for image contrast. Image contributed by Sima Zacharek, Department of Genetics, Children's Hospital Stem Cell Program, Children's Hospital Boston, Massachusetts.

reaction. Some fluorophores are not completely compatible with the copper that drives the Click-iT™ reaction, requiring adjustments to the workflow (Table 3). In most cases, antibodies, lectins, fluorescent proteins (Figures 6 and 7), or cell cycle stains can be used with Click-iT™ EdU for deeper biological insight.

Click chemistry holds enormous potential for a variety of applications. Find out what Click-iT™ EdU can do for your cell proliferation assays at **www.invitrogen.com/edu**. ■

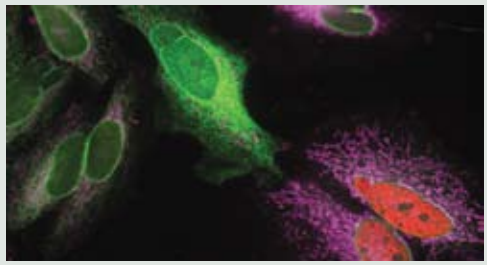

Figure 7—Click-iT™ EdU compatibility with fluorescent proteins. HeLa cells were transduced with Organelle Lights™ NE GFP (Cat. no. O36213) for 16 hours, then incubated for 60 minutes with reagents from the Click-iT™ EdU Alexa Fluor® 594 Imaging Kit (Cat. no. C10084) to detect proliferating cells (red fluorescence) and with MitoTracker® Deep Red FM (Cat. no. M22426) to detect mitochondria (pseudocolored pink). Following fixation and permeabilization, green fluorescent protein (GFP) expressed in the nuclear envelope was detected with rabbit anti-GFP serum (Cat. no. A6455) and visualized using Alexa Fluor® 488 goat anti–rabbit IgG antibody (Cat. no. A11008). Green fluorescence is also seen in the endoplasmic reticulum as it forms from the nuclear envelope.

References

1. Breinbauer, R. and Köhn, M. (2003) *ChemBioChem* 4:1147–1149.
2. Wang, Q. et al. (2003) *J Am Chem Soc* 125:3192–3193.
3. Rostovtsev, V.V. et al. (2002) *Angew Chem Int Ed Engl* 41:2596–2599.
4. Kolb, H.C. et al. (2001) *Angew Chem Int Ed Engl* 40:2004–2021.

Table 3—Click-iT™ EdU compatibility.*

Fluorescent molecules	Notes
Qdot® nanocrystals	Use Qdot® nanocrystals after the Click-iT™ detection reaction
Fluorescent proteins (GFP)	Use organic dye–labeled expression tags (e.g., TC FlAsH/ReAsH) to detect protein expression; use anti-GFP antibodies before or after the Click-iT™ detection reaction to detect and generate fluorescence †
Organic dyes (e.g., Alexa Fluor® dyes, fluorescein (FITC))	Completely compatible with Click-iT™ EdU
PerCP, allophycocyanin (APC) and APC-based tandems (e.g., Alexa Fluor® 680-APC)	Completely compatible with Click-iT™ EdU
R-phycoerythrin (RPE) and RPE-based tandem conjugates (e.g., Alexa Fluor® 610-RPE)	Use RPE and RPE-based tandem conjugates after the Click-iT™ detection reaction

* Compatibility indicates whether the fluorescent molecule itself or the detection methods involve components that are unstable in the presence of the copper catalyst used for the Click-iT™ detection reaction. † Not all anti-GFP antibodies recognize the same antigen site. Rabbit and chicken anti-GFP antibodies perform well, whereas mouse monoclonal antibodies do not generate an acceptable amount of fluorescence and are not recommended for this application.

Product	Quantity	Cat. no.
Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay Kit, 10-plate size	1 kit	C10081
Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay Kit, 10-plate size	1 kit	C10082
Click-iT™ EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10027
Click-iT™ EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay Kit, 10-plate size	1 kit	A10028
Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10209
Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10208
Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay, 50 assays	1 kit	A10034
Click-iT™ EdU Alexa Fluor® 488 Flow Cytometry Assay, 50 assays	1 kit	C35002
Click-iT™ EdU Alexa Fluor® 647 Flow Cytometry Assay, 50 assays	1 kit	A10202
Click-iT™ EdU Alexa Fluor® 488 Imaging Assay Kit, 50 coverslips	1 kit	C10083
Click-iT™ EdU Alexa Fluor® 594 Imaging Assay Kit, 50 coverslips	1 kit	C10084
Click-iT™ EdU Alexa Fluor® 647 Imaging Assay Kit, 50 coverslips	1 kit	C10085
EdU (5-ethynyl-2'-deoxyuridine)	50 mg	A10044