molecular probes by life technologies

Click-iT® EdU Imaging Kit with Alexa Fluor® 488, 594, and 647 Azides

Cat. no. C10086

Click-iT® EdU cell proliferation assays eliminate DNA denaturation steps required by BrdU assays, steps that can damage sample morphology and integrity. This trial-size kit provides sufficient EdU to label up to 6 coverslips and enough Alexa Fluor® 488, 594, and 647 azide to detect new DNA synthesis on 2 coverslips with each fluorophore (Figure 1).

Kit contents:

- EdU (5-ethynyl-2′-deoxyuridine, purple cap)
- Alexa Fluor® 488 azide (green cap)
- Alexa Fluor® 594 azide (orange cap)
- Alexa Fluor® 647 azide (red cap)
- $CuSO_4$ (large bttle, clear cap)
- Click-iT® EdU reaction buffer concentrate (blue cap)
- Click-iT® EdU buffer additive (white cap)

Required but not included:

- PBS
- 0.5% Triton® X-100 in PBS
- 3% BSA in PBS
- 3.7% paraformaldehyde in PBS
- Deionized water or 18 megohm purified water
- Complete medium
- 6-well plate
- 18 × 18 mm sterile coverslips

Click-iT® EdU compatibility

Fluorescent molecule	Notes
Qdot® nanocrystals	Use Qdot® nanocrystals after the Click-iT® detection reaction
Fluorescent proteins (GFP)	Use anti-GFP antibodies* before the Click-iT® detection reaction or Use organic dye-based reagents for protein expression detection
Organic dyes (i.e., Alexa Fluor® dyes, fluorescein (FITC))	These fluorescent molecules are completely compatible with Click-iT® EdU
TC-FlAsH™ / TC-ReAsH™ reagents	Detect the tetracysteine (TC) tag before 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 tested do not generate an acceptable amount of fluorescence, and are not recommended for this application.

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Experimental protocol

Preparing cells

1.1 Plate cells on coverslips at desired density and allow to recover overnight before additional treatment.

1.2 Treat cells as desired.

Labeling cells with 10 µM EdU

2.1 Transfer coverslips into the 6-well plate already containing medium so that each well contains a single coverslip.

2.2 Prepare a 20 µM solution of EdU in prewarmed complete medium. The EdU is provided as a 10 mM solution in DMSO.

2.3 Add an equal volume of the 20 µM EdU solution to the volume of medium containing the cells to obtain a 10 µM solution.

2.4 Incubate for 15–60 minutes.

Cell fixation and permeabilization

3.1 Remove medium and add 1 mL 3.7% paraformaldehyde in PBS (fixative) to each well.

3.2 Incubate for 15 minutes at room temperature.

3.3 Remove fixative and wash cells 2X with 1 mL 3% BSA in PBS (wash solution).

3.4 Remove wash solution. Add 1 mL 0.5% Triton® X-100 in PBS (permeabilization buffer) to each well.

3.5 Incubate for 20 minutes.

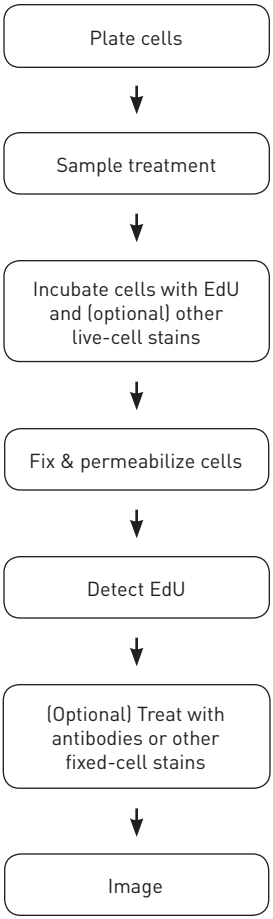
EdU detection for 2 coverslips with Alexa Fluor® dye of choice

4.1 Prepare 1X Click-iT® EdU buffer additive by adding 1.0 mL of dH_2O to white-capped vial.

4.2 Prepare Click-iT® reaction cocktail by adding the following amounts to the Alexa Fluor® amber vial:

- 100 µL Click-iT® reaction buffer (blue-capped vial)
- 800 µL $CuSO_4$ (large clear bottle)
- 100 µL 1X Click-iT® reaction buffer additive (white-capped vial; prepared in step 4.1)

Use this within 15 minutes after preparation.



4.3 Remove permeabilization buffer and wash cells 2X with 1 mL 3% BSA in PBS, then remove the wash solution.

4.4 Add 0.5 ml Click-iT® reaction cocktail to each well. Rock plate to evenly distribute solution.

4.5 Incubate for 30 minutes, protected from light.

4.6 Remove Click-iT® reaction cocktail and wash 2X with 1 mL 3% BSA in PBS.

Antibody and DNA staining (optional)

5.1 Stain with primary and secondary antibodies.

5.2 Stain with DNA counterstain.

Imaging

6.1 Use filters appropriate for the Alexa Fluor® dye, live-cell stains, secondary antibodies, and DNA counterstain (Figures 2–5).

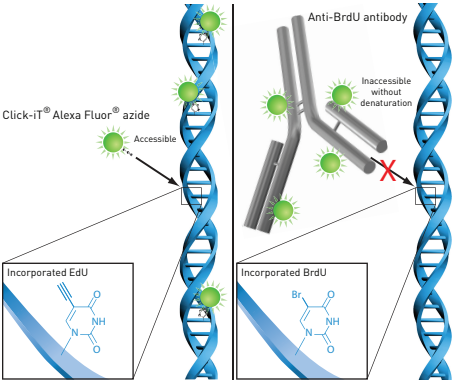


Figure 1—Detection of the incorporated EdU with the Alexa Fluor® 488 azide versus incorporated BrdU with an anti-BrdU antibody. The small size of the Alexa Fluor® 488 azide eliminates the need to denature DNA in order for the detection reagent to gain access to the modified base.

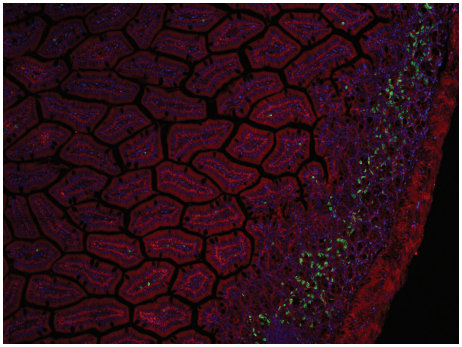


Figure 2—Click-iT® EdU: Amazing imagery following EdU administration *in vivo*. Mice were injected with EdU at 0.3 mg /10 g mouse weight. An intestinal section was stained with green fluorescent Click-iT® EdU Alexa Fluor® 488 Imaging Kit (C10337) and cells that incorporated EdU were visualized with green fluorescence. Nuclei are counterstained with blue fluorescent DAPI (D1306) and red-fluorescence is from autofluorescence.

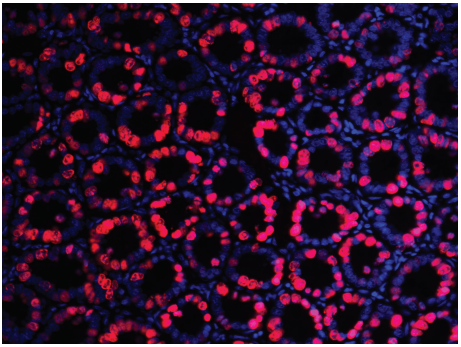


Figure 3—Eliminate long detection procedures with Click-iT® EdU. Rat Ileum tissue section detected with red-fluorescent Click-iT® EdU Alexa Fluor® 594 Imaging Kit (C10339). EdU staining complete in 80 minutes, while BrdU protocols require harsh permeabilization and overnight anti-BrdU detection. Nuclei are counterstained with blue fluorescent Hoechst 33342 (H1399).

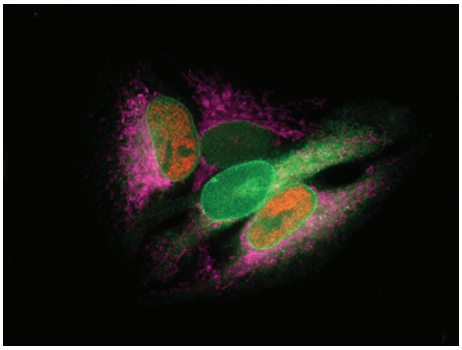


Figure 4—Click-iT® EdU compatibility with fluorescent proteins and other live-cell stains. HeLa cells transduced with Organelle Lights™ NE GFP prior to being incubated with EdU and MitoTracker® Deep Red FM (M22426). GFP expressed in the nuclear envelope was detected with anti-green fluorescent protein, rabbit serum (A6455) and visualized with an Alexa Fluor® 488 goat anti-rabbit IgG antibody (A11034). Green fluorescence is also seen in the endoplasmic reticulum as it is formed from the nuclear envelope. Proliferating cells were detected with a Click-iT® EdU Alexa Fluor® 594 Imaging Kit (red-fluorescence, C10339). Mitochondria are pseudocolored pink.

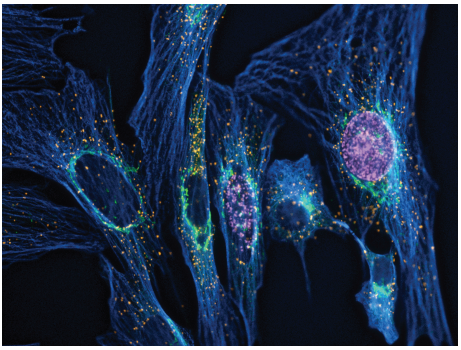


Figure 5—Click-iT® EdU requires mild fixation; enabling truly spectacular multicolor imagery. Muntjac cells were treated with 10 µM EdU. EdU incorporated into newly synthesized DNA was detected by the far red-fluorescent Click-iT® EdU Alexa Fluor® 647 Imaging Assay (C10340). Tubulin was labeled with anti-tubulin antibody (A11126) and visualized with Alexa Fluor® 350 goat anti-mouse IgG antibody (A21049). The Golgi complex was stained with the green-fluorescent Alexa Fluor® 488 conjugate of lectin HPA from *Helix pomatia* (edible snail; L11271), and peroxisomes were labeled with an anti-peroxisome antibody and visualized with an orange-fluorescent Alexa Fluor® 555 donkey anti-rabbit IgG antibody (A31572).

Ordering information

Product	Quantity	Cat. no.
Click-iT® EdU Imaging Kit with Alexa Fluor® 488, 594, and 647 Azides	1 kit	C10086
Click-iT® EdU Alexa Fluor® 488 Imaging Kit *for 50 coverslips*	1 kit	C10337
Click-iT® EdU Alexa Fluor® 555 Imaging Kit *for 50 coverslips*	1 kit	C10338
Click-iT® EdU Alexa Fluor® 594 Imaging Kit *for 50 coverslips*	1 kit	C10339
Click-iT® EdU Alexa Fluor® 647 Imaging Kit *for 50 coverslips*	1 kit	C10340
EdU (5-ethynyl-2'-deoxyuridine)	50 mg	A10044

For more information, please visit www.lifetechnologies.com/edu.

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