GeneCopoeia™
Expressway to Discovery

9620 Medical Center Drive, Suite 101
Rockville, MD 20850, USA
www.genecopoeia.com
1-866-360-9531

iClick™ EdU Andy Fluor 488 Imaging Kit
Catalog Number: A003

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
EdU (Component A)	**2 × 1 mL**	**10 mM in DMSO**	**-20 °C**	**The product is stable for at least one year when stored as directed.**
Andy Fluor 488 azide (Component B)	**100 µL**	**NA**	**-20 °C Protect from light**	
iClick EdU reaction buffer (Component C)	**50 mL**	**1X**	**4 °C**	
$CuSO_4$ (Component D)	**1 mL**	**100 mM in H_2O**	**4 °C**	
iClick EdU buffer additive (Component E)	**200 mg**	**NA**	**4 °C**	
Hoechst 33342 (Component F)	**70 µL**	**5 mg/mL in H_2O**	**4 °C**	

Number of assays: Sufficient material is supplied for 50 coverslips based on the protocol below.
Approximate fluorescence excitation/emission maxima, in nm: Andy Fluor 488 azide**:** 495/520; Hoechst 33342: 350/461, bound to DNA.

Introduction

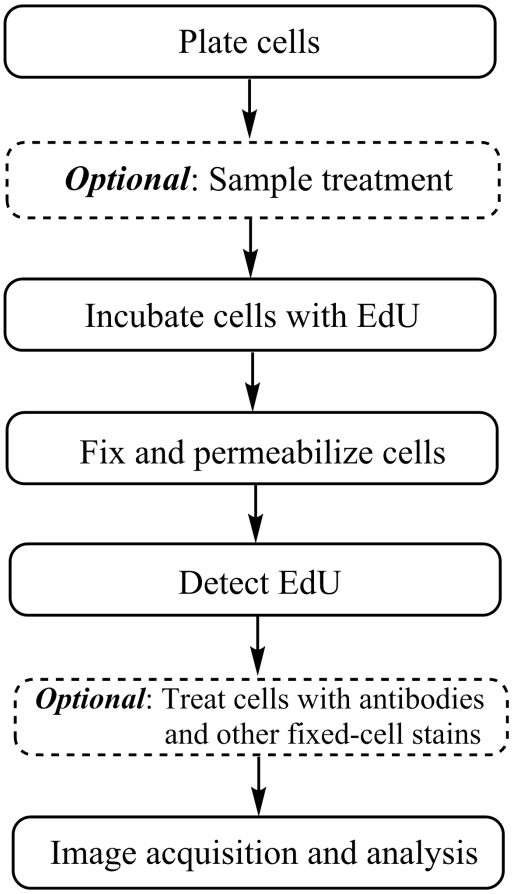
Detecting cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drugs. The most accurate method of doing this is by directly measuring DNA synthesis. Usually this was performed by antibody-based detection of the nucleoside analog bromo-deoxyuridine (BrdU). The iClick™ EdU Assay is a novel alternative to the BrdU assay. EdU (5-ethynyl-2´-deoxyuridine) provided in the kit is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis. Detection is based on a click reaction, a copper-catalyzed covalent reaction between an azide and an alkyne. In this application, the EdU contains the alkyne and the Andy Fluor 488 dye contains the azide. The advantages of the iClick EdU labeling are readily evident while performing the assay. The small size of the dye azide allows for efficient detection of the incorporated EdU using mild conditions. Standard aldehyde-based fixation and detergent permeabilization are sufficient for the iClick detection reagent to gain access to the DNA. This is in contrast to BrdU assays that require DNA denaturation (typically using HCl or heat or digestion with DNase) to expose the BrdU so that it may be detected with an anti-BrdU antibody.

The kit contains all of the components needed to label and detect the incorporated EdU as well as perform cell cycle analysis on samples from adherent cells. For cell cycle analysis, the kit is supplied with blue fluorescent Hoechst 33342 dye. The kit includes sufficient reagents for labeling 50, 18 × 18 coverslips using 500 µL of reaction buffer per test.

Materials required but not provided

- Phosphate-buffered saline (PBS, pH 7.2–7.6)
- Fixative (3.7% Formaldehyde in PBS)
- Permeabilization reagent (0.5% Triton® X-100 in PBS)
- 3% Bovine serum albumin (BSA) in PBS (3% BSA in PBS), pH 7.4
- Deionized water
- 18 × 18-mm coverslips

Workflow diagram for the iClick EdU Imaging Assay



Experimental Protocols

Labeling cells with EdU

Note: The optimal EdU concentration varies with different cell types. It is recommended to start with EdU concentration at 10 μM. Growth medium, cell density, cell type variations, and other factors may influence labeling. In initial experiments, we recommend testing a range of EdU concentrations to determine the optimal concentration for your cell type and experimental conditions.

1.1 Plate the cells on coverslips at the desired density, then allow them to recover overnight before additional treatment.

1.2 Prepare a 2X working solution of EdU (Component A) in complete medium from the 10 mM stock solution. A suggested starting concentration is 10 μM.

1.3 Prewarm the 2X EdU solution, then add an equal volume of the 2X EdU solution to the volume of media containing cells to be treated to obtain a 1X EdU solution. (For example, for a final concentration of 10 μM, replace half of the media with fresh media containing 20 μM of EdU). We do not recommend replacing all of the media, because this could affect the rate of cell proliferation.

1.4 Incubate the cells for the desired length of time under conditions optimal for the cell type. The time of EdU exposure to the cells allows for direct measurement of cells synthesizing DNA. The choice of time points and the length of time depend on the cell growth rate. Pulse labeling of cells by brief exposures to EdU permits studies of cell-cycle kinetics.

Cell fixation and permeabilization

Note: This protocol is optimized with a fixation step using 3.7% formaldehyde in PBS, followed by a 0.5% Triton® X-100 permeabilization step. However, this protocol is also amenable to other fixation/ permeabilization reagents, such as methanol and saponin.

2.1 After incubation, remove the media and add 1 mL of 3.7% formaldehyde in PBS to each well containing the coverslips. Incubate for 15 minutes at room temperature.

2.2 Remove the fixative and wash the cells in each well twice with 1 mL of 3% BSA in PBS.

2.3 Remove the wash solution. Add 1 mL of 0.5% Triton® X-100 in PBS to each well, then incubate at room temperature for 20 minutes.

EdU detection

Note: This protocol uses 500 µL of iClick reaction cocktail per coverslip. A smaller volume can be used as long as the remaining reaction components are maintained at the same ratios.

3.1 Make a 10X stock solution of the iClick EdU buffer additive (Component E): Add 1 mL of deionized water to the vial, then mix until fully dissolved. After use, store any remaining stock solution at ≤–20°C. When stored as directed, this stock solution is stable for up to 1 year. If the solution develops a brown color, it has degraded and should be discarded.

3.2 Prepare 1X iClick EdU buffer additive by diluting the 10X solution 1:10 in deionized water. Prepare this solution **fresh** and use the solution on the same day.

3.3 Prepare iClick reaction cocktail according to Table 2. It is important to add the ingredients in the order listed in the table; otherwise, the reaction will not proceed optimally. Use the iClick reaction cocktail within 15 minutes of preparation.

Table 2. iClick reaction cocktails.

Reaction components	Number of coverslips			
	1	2	4	10
iClick EdU reaction buffer (Component C)	430 µL	860 µL	1.8 mL	4.3 mL
$CuSO_4$ (Component D)	20 µL	40 µL	80 µL	200 µL
Andy Fluor 488 azide (Component B)	1.5 µL	3 µL	6 µL	15 µL
1X Reaction buffer additive (prepared in step 3.2)	50 µL	100 µL	200 µL	500 µL
Total volume	500 µL	1 mL	2 mL	5 mL

3.4 Remove the permeabilization buffer, then wash the cells in each well twice with 1 mL of 3% BSA in PBS. Remove the wash solution.

3.5 Add 0.5 mL of iClick reaction cocktail to each well containing a coverslip. Rock the plate briefly to insure that the reaction cocktail is distributed evenly over the coverslip.

3.6 Incubate the plate for 30 minutes at room temperature, **protected from light**.

3.7 Remove the reaction cocktail, then wash each well once with 1 mL of 3% BSA in PBS. Remove the wash solution.

For nuclear staining, proceed to **DNA staining**. If no additional staining is desired, proceed to **Imaging and analysis**.

DNA staining

4.1 Wash each well with 1 mL of PBS. Remove the wash solution.

4.2 Dilute the Hoechst 33342 (Component F) solution 1:1000 in PBS to obtain a 1X Hoechst 33342 solution (the final concentration is 5 µg/mL).

4.3 Add 1 mL of 1X Hoechst 33342 solution per well. Incubate for 15 minutes at room temperature, **protected from light**. Remove the Hoechst 33342 solution.

4.4 Wash each well twice with 1 mL of PBS. Remove the wash solution.

Imaging and analysis

iClick EdU cells are compatible with all methods of slide preparation, including wet mount or prepared mounting media. See Table 3 for the approximate fluorescence excitation/emission maxima for Andy Fluor 488 dye and Hoechst 33342 dye bound to DNA.

Table 3. Approximate fluorescence excitation/emission maxima.

Fluorophore	Excitation (nm)	Emission (nm)
Andy Fluor 488	495	520
Hoechst 33342, bound to DNA	350	461

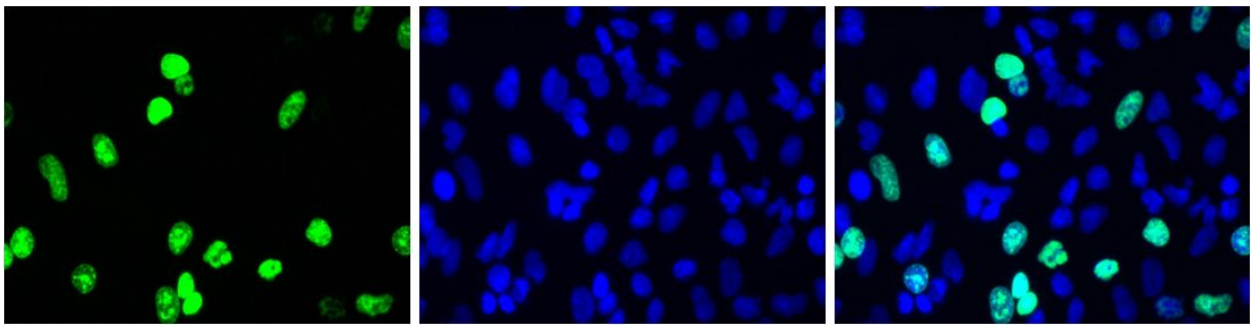


Figure 1. A549 cells was treated with 10 mM EdU for 2 h, followed by click detection with Andy Fluor™ 488 Azide (green), nuclei are counterstained with Hoechst 33342 (blue).