

## iClick™ EdU Andy Fluor 647 Flow Cytometry Assay Kit

Catalog Number: A008

**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 647 azide (Component B)	150 µL	NA	-20 °C Protect from light	
iClick fixative (Component C)	5 mL	1X solution	4 °C	
iClick permeabilization and wash reagent (Component D)	50 mL	10X solution	4 °C	
CuSO <sub>4</sub> (Component E)	1 mL	100 mM in H <sub>2</sub> O	4 °C	
iClick EdU buffer additive (Component F)	200 mg	NA	4 °C	

**Number of assays:** Sufficient material is supplied for 50 reactions based on the protocol below.

**Approximate fluorescence excitation/emission maxima, in nm:** Andy Fluor 647 azide: 650/665.

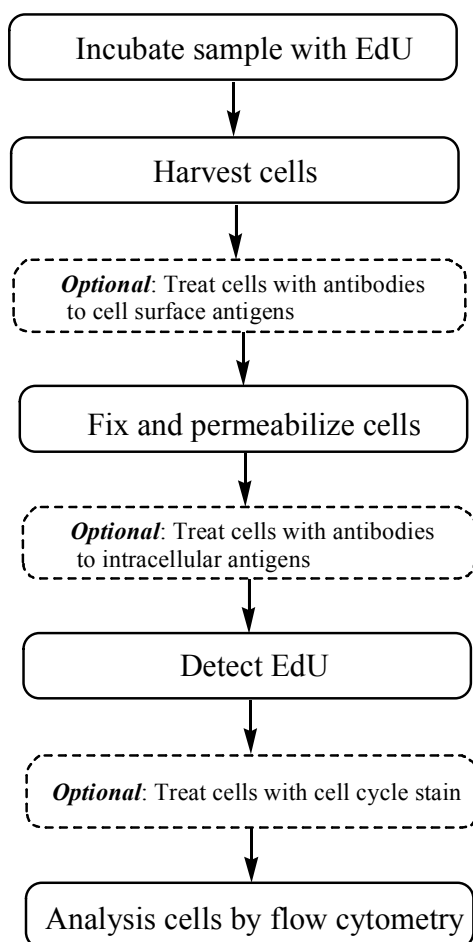
### Introduction

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 bromodeoxyuridine (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 647 dye contains the azide. The advantages of the Click-iT 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 Click-iT 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. Sample processing for the BrdU assay can result in signal alteration of the cell cycle distribution as well as the destruction of antigen recognition sites when using the acid denaturation method. In contrast, the EdU cell proliferation kit is compatible with cell cycle dyes. The EdU assay can also be multiplexed with antibodies against surface and intracellular markers.

### Materials required but not provided

- Buffered saline solution, such as PBS, D-PBS, or TBS
- 1% Bovine serum albumin (BSA) in PBS (1% BSA in PBS), pH 7.4
- Deionized water
- 12 × 75-mm tubes, or other flow cytometry tubes

### Workflow diagram for the iClick EdU Flow Cytometry 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  $\mu$ M. 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** Suspend the cells in an appropriate tissue culture medium to obtain optimal conditions for cell growth. Disturbing the cells by temperature changes or washing prior to incubation with EdU slows the growth of the cells during incorporation.
- 1.2** Add EdU to the culture medium at the desired final concentration and mix well. We recommend a starting concentration of 10  $\mu$ M for 1–2 hours. For longer incubations, use lower concentrations. For shorter incubations, higher concentrations may be required. For a negative staining control, include cells from the same population that have not been treated with EdU.
- 1.3** Incubate under conditions optimal for cell type for the desired length of time. Altering the amount of time the cells are exposed to EdU or subjecting the cells to pulse labeling with EdU allows the evaluation of various DNA synthesis and proliferation parameters. Effective time intervals for pulse labeling and the length of each pulse depend on the cell growth rate.

#### 1.4 Harvest cells.

### Staining Cell-Surface Antigens with Antibodies (Optional)

2.1 Wash cells once with 3 mL of 1% BSA in PBS, pellet cells by centrifugation, and remove supernatant.

2.2 Dislodge the pellet and resuspend cells at  $1 \times 10^7$  cells/mL in 1% BSA in PBS.

2.3 Add 100  $\mu$ L of cell suspension to flow tubes.

2.4 Add surface antibodies and mix well.

2.5 Incubate for the recommended time and temperature, protected from light.

### Cell fixation and permeabilization

**Note:** The saponin-based permeabilization reagent can be used with whole blood or cell suspensions containing red blood cells, as well as with cell suspensions containing more than one cell type. This permeabilization reagent maintains the morphological light scatter characteristics of leukocytes while lysing red blood cells.

3.1 Wash the cells once with 3 mL of 1% BSA in PBS, pellet the cells, and remove the supernatant.

3.2 Dislodge the pellet, add 100  $\mu$ L of iClick fixative (Component C), and mix well.

3.3 Incubate the cells for 15 minutes at room temperature, protected from light.

3.4 Wash the cells with 3 mL of 1% BSA in PBS, pellet the cells, and remove the supernatant.

3.5 Dislodge the cell pellet and resuspend the cells in 100  $\mu$ L of 1X iClick permeabilization and wash reagent (prepared by adding 50 mL of component D to 450 mL of 1% BSA in PBS), and mix well. Incubate the cells for 20 minutes.

### EdU detection

**Note:** This protocol uses 500  $\mu$ L of iClick reaction cocktail. A smaller volume can be used as long as the remaining reaction components are maintained at the same ratios.

4.1 Make a 10X stock solution of the iClick EdU buffer additive (Component F): Add 1 mL of deionized water to the vial, then mix until fully dissolved. After use, store any remaining stock solution at  $\leq -20^\circ\text{C}$ . 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.

4.2 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.

4.3 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.** Click-iT reaction cocktails.

Reaction components	Number of reactions			
	1	2	5	10
PBS, D-PBS, or TBS	438 $\mu$ L	875 $\mu$ L	2.19 mL	4.38 mL
CuSO <sub>4</sub> (Component E)	10 $\mu$ L	20 $\mu$ L	50 $\mu$ L	100 $\mu$ L
Andy Fluor 647 azide (Component B)	2.5 $\mu$ L	5 $\mu$ L	12.5 $\mu$ L	25 $\mu$ L
1X Reaction buffer additive (prepared in step 4.2)	50 $\mu$ L	100 $\mu$ L	250 $\mu$ L	500 $\mu$ L
Total volume	500 $\mu$ L	1 mL	2.5 mL	5 mL

4.4 Add 0.5 mL of Click-iT reaction cocktail to each tube and mix well.

4.5 Incubate the reaction mixture for 30 minutes at room temperature, protected from light.

**4.6** Wash the cells once with 3 mL of 1X iClick permeabilization and wash reagent, pellet the cells, and remove the supernatant.

### **Staining Intracellular or Surface Antigens (Optional)**

**5.1** Dislodge the cell pellet and resuspend the cells in 100  $\mu$ L of 1X iClick permeabilization and wash reagent.

**5.2** Add antibodies against intracellular antigens or against surface antigens. Mix well.

**5.3** Incubate the tubes for the time and temperature required for antibody staining, protected from light.

**5.4** Wash each tube with 3 mL of 1X iClick permeabilization and wash reagent, pellet the cells, and remove the supernatant.

### **Staining Cells for DNA Content (Optional)**

**6.1** Dislodge the cell pellet and resuspend the cells in 500  $\mu$ L of 1X iClick permeabilization and wash reagent.

**6.2** If necessary, add Ribonuclease A to each tube and mix.

**6.3** Add the appropriate DNA stain to each tube, mix well, and incubate as recommended for each DNA stain.

### **Analysis by Flow Cytometry**

If measuring total DNA content on a traditional flow cytometer using hydrodynamic focusing, use a low flow rate during acquisition. The fluorescent signal generated by DNA content stains is best detected with linear amplification. The fluorescent signal generated by iClick EdU labeling is best detected with logarithmic amplification.

**7.1** Analyze the cells using a flow cytometer. For the detection of EdU with Andy Fluor 647 azide, use 633/635 nm excitation with a red emission filter (660/20 nm or similar).