LIFESCT
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# LiFluor™ 647 EdU Imaging Kit

Cat. #: C0019 Size: 50 rxns

## Introduction

***LiFlour™ 647 EdU Imaging 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.

## Package Information

| Components | C0019 |
|---|---|
| EdU* | 2×1 ml, 10 mM in DMSO |
| LiFluor 647 azide* | 100 µl |
| EdU reaction buffer | 50 ml |
| EdU buffer additive | 200 mg |
| $CuSO_4$ | 1 ml, 100 mM in $H_2O$ |
| Hoechst 33342 | 70 µl, 5 mg/ml in $H_2O$ |

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

Approximate fluorescence excitation/emission maxima, in nm: LiFlour 647 azide: 650/665; Hoechst 33342: 350/461, bound to DNA.

## Storage

*Store at -20°C, Protect from light; Other at 4°C

## Materials required but not provided

**1.** Phosphate-buffered saline (PBS, pH 7.2–7.6)
2. Fixative (3.7% Formaldehyde in PBS)
3. Permeabilization reagent (0.5% Triton® X-100 in PBS)
4. 3% Bovine serum albumin (BSA) in PBS (3% BSA in PBS), pH 7.4
5. Deionized water
6. 18× 18-mm coverslips

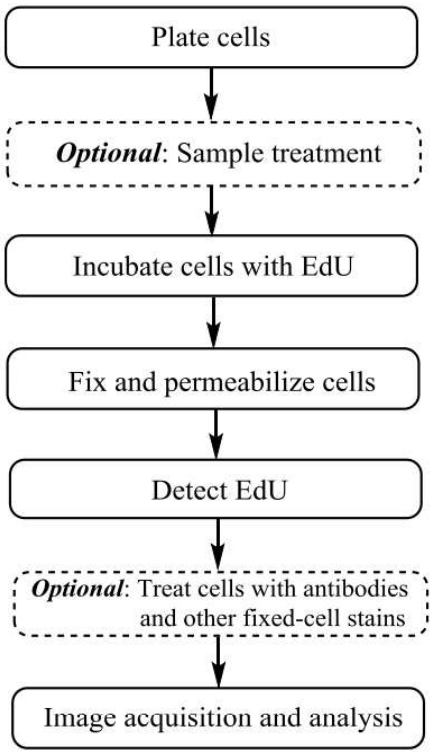
## Protocols

### 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.

Workflow diagram for the LiFlour EdU Assay




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

1.3 Prewarm the 2× EdU solution, then add an equal volume of the 2× EdU solution to the volume of media containing cells to be treated to obtain a 1× 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.

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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 LiFluor 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 10× stock solution of the EdU buffer additive: 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 1× EdU buffer additive (see Table 1) by diluting the 10× solution 1:10 in deionized water. Prepare this solution fresh and use the solution on the same day.

3.3 Prepare LiFluor reaction cocktail according to Table 1. It is important to add the ingredients in the order listed in the table; otherwise, the reaction will not proceed optimally. Use the LiFuor reaction cocktail within 15 minutes of preparation.

**Table 1**. LiFlour reaction cocktails

| Reaction components | Number of coverslips | | | |
|---|---|---|---|---|
| | 1 | 2 | 4 | 10 |
| EdU reaction buffer | 430 µl | 860 µl | 1.8 ml | 4.3 ml |
| $CuSO_4$ | 20 µl | 40 µl | 80 µl | 200 µl |
| LiFluor 647 azide | 1.5 µl | 3 µl | 6 µl | 15 µl |
| 1× Reaction buffer additive (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 LiFuor 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 solution 1:1000 in PBS to obtain a 1× Hoechst 33342 solution (the final concentration is 5 µg/ml).

4.3 Add 1 ml of 1× Hoechst 33342 solution per well. Incubate for 30 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

LiFluor EdU cells are compatible with all methods of slide preparation, including wet mount or prepared mounting media. See Table 2 for the approximate fluorescence excitation/emission maxima for Liluor 647 dye and Hoechst 33342 dye bound to DNA.

**Table 2**. Approximate fluorescence excitation/emission maxima

| Fluorophore | Excitation (nm) | Emission (nm) |
|---|---|---|
| LiFluor 647 | 650 | 665 |
| Hoechst 33342, bound to DNA | 350 | 461 |