LIFESCT
Life Scientists' Service Center

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LiFluor™ 488 EdU Flow Cytometry Assay Kit

Cat. #: C0020 Size: 50 rxns

Introduction

LiFlour™ 488 EdU Flow Cytometry Assay Kit 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 LiFlour™ 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 LiFlour™ 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.

Package Information

Components	C0020
EdU*	2×1 ml, 10 mM in DMSO
LiFluor 488 azide*	150 µl
LiFlour fixative	5 ml, 1×
Permeabilization and wash reagent	50 ml, 10×
$CuSO_4$	1 ml, 100 mM in H_2O
EdU buffer additive	200 mg

Number of assays: Sufficient material is supplied for 50 reactions based on the protocol below.
Approximate fluorescence excitation/emission maxima, in nm: LiFlour 488 azide: 495/520

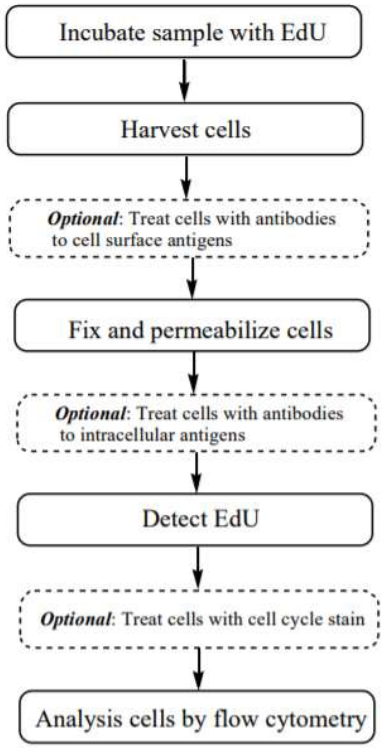
Storage

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

Materials required but not provided

1. Buffered saline solution, such as PBS, D-PBS, or TBS
2. 1% Bovine serum albumin (BSA) in PBS (1% BSA in PBS), pH 7.4
3. Deionized water
4. 12× 75-mm tubes, or other flow cytometry tubes

Workflow diagram for the LiFlour EdU Flow Cytometry Assay



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 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 µM 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.

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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 × 10^7 cells/ml in 1% BSA in PBS.

2.3 Add 100 µl 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 µl of LiFlour fixative, 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 µl of 1× permeabilization and wash reagent (prepared by adding 50 ml of permeabilization and wash reagent to 450 ml of 1% BSA in PBS), and mix well. Incubate the cells for 20 minutes.

EdU detection

Note: This protocol uses 500 µl of LiFour 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 10× stock solution of the LiFlour 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.

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

4.3 Prepare LiFlour 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 LiFlour reaction cocktail within 15 minutes of preparation.

Table 1. LiFlour reaction cocktails

Reaction components	Number of reactions			
	1	2	5	10
PBS, D-PBS, or TBS	438 µl	875 µl	2.19 ml	4.38 ml
$CuSO_4$	10 µl	20 µl	50 µl	100 µl
LiFluor 488 azide	2.5 µl	5 µl	12.5 µl	25 µl
1× EdU buffer additive	50 µl	100 µl	250 µl	500 µl
Total volume	500 µl	1 ml	2.5 ml	5 ml

4.4 Add 0.5 ml of LiFlour 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 1× LiFlour 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 µl of 1× 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 1× 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 µl of 1× 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 LiFlour EdU labeling is best detected with logarithmic amplification.

7.1 Analyze the cells using a flow cytometer. For the detection of EdU with LiFluor 488 azide, use 488 nm excitation with a green emission filter (530/30 nm or similar).