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LiFluor™ 488 EU Imaging Kit

Cat. #: C0022 Size: 50 rxns

Introduction

LiFluor™ 488 EU Imaging Kit contains all of the components needed to label and detect newly synthesized RNA in whole cells. The kits are also supplied with blue fluorescent Hoechst 33342 dye as a nuclear counterstain or for DNA profiling. The kits include sufficient reagents for labeling 50, 18× 18 mm coverslips using 500 µl reaction volume per well.

Package Information

Components	C0022
EU*	1 ml (50 mM in DMSO)
LiFluor 488 azide*	100 µl
EU reaction buffer	50 ml
$CuSO_4$	1 ml (100 mM in H_2O)
EU buffer additive	200 mg
Hoechst 33342	70 µl (5 mg/ml in H_2O)

Number of assays: Sufficient material is supplied for 50 coverslips based on the protocol below.
Approximate fluorescence excitation/emission maxima, in nm: LiFlour 488 azide: 590/615; 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

Protocols

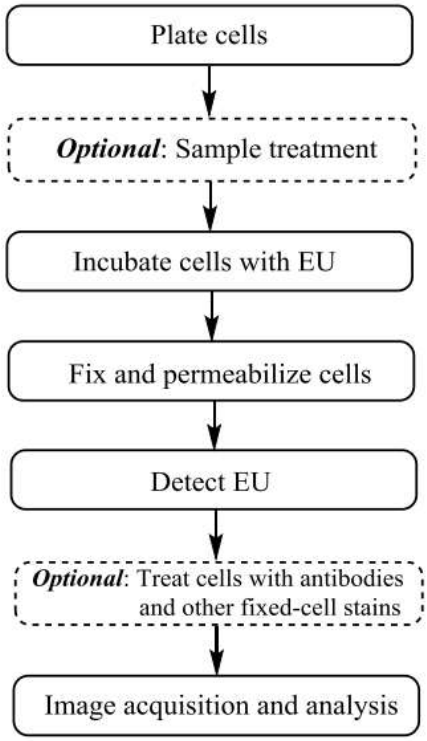
Labeling cells with EU

Note: The optimal EU concentration varies with different cell types. It is recommended to start with EU concentration at 0.5 mM. Growth medium, cell density, cell type variations, and other factors may influence labeling. In initial experiments, we recommend testing a range of EU 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 Optional: Perform cell treatments as desired. To address the potential reversibility of drug action on RNA synthesis, we recommend that you do not remove the drug-containing media during EU treatment.

Workflow diagram for the LiFlour EU Imaging Assay



1.3 Prepare a 2× working solution of EU from the 50 mM stock solution in pre-warmed complete medium. For example, for a 0.5 mM final EU treatment, prepare a 1 mM working solution.

1.4 Prewarm the 2× EU solution, then add an equal volume of the 2× EU solution to the volume of media containing cells to be treated to obtain a 1× EU solution. (For example, for a final concentration of 0.5 mM, replace half of the media with fresh media containing 1 mM of EU). We do not recommend replacing all of the media, because this could affect the rate of cell proliferation.

1.5 Incubate under normal cell culture conditions for 1 hour. The optimal incubation time depends on the cell growth rate and cell treatment, and needs to be determined experimentally.

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 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 EU 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× EU buffer additive 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 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 reaction cocktail within 15 minutes of preparation.

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 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.

Table 1. LiFlour reaction cocktails

Reaction components	Number of coverslips			
	1	2	4	10
EU reaction buffer	430 µl	860 µl	1.8 ml	4.3 ml
$CuSO_4$	20 µl	40 µl	80 µl	200 µl
LiFluor 488 azide	2 µl	4 µl	8 µl	20 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

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 EU 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 LiFluor 594 dye and Hoechst 33342 dye bound to DNA.

Table 2. Approximate fluorescence excitation/emission maxima

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