EdU Imaging Kits (488)

Protocol

Preparing reagents

1.1 Allow vials to warm to room temperature before opening.

1.2 To prepare a 10 mM solution of EdU, add 2 mL of DMSO (Component C) or aqueous solution(buffer, PBS, or saline) to Component A and mix well. After use, store any remaining stock solution at ≤–20°C. When stored as directed, the stock solution is stable for up to 1 year.

1.3 Prepare a working solution of 1X EdU reaction buffer (Component D): Transfer all of the solution (4 mL) in the Component D bottle to 36 mL of deionized water. Rinse the Component D bottle with some of the diluted 1X EdU reaction buffer to ensure the transfer of all of the 10X concentrate.

To make smaller amounts of 1X EdU reaction buffer, dilute volumes from the Component D bottle 1:10 with deionized water. After use, store any remaining 1X solution at 2–6˚C. When stored as directed, this 1X solution is stable for up to 6 months.

1.4 To make a 10X stock solution of the EdU buffer additive (Component F), add 2 mL of deionized water to the vial and mix until the EdU buffer additive is fully dissolved. After use, store any remaining stock solution at ≤–20˚C. When stored as directed, the stock solution is stable for up to 1 year. If the solution develops a brown color, it has degraded and should be discarded.

Materials required but not provided

A. Phosphate-buffered saline (PBS, pH 7.2–7.6)

B. Fixative (for example, 3.7% Formaldehyde in PBS)

C. Permeabilization reagent (for example, 0.3% Triton® X-100 in PBS)

D. 3% Bovine serum albumin (BSA) in PBS (3% BSA in PBS), pH 7.4

E. Deionized water

F. 18 × 18-mm coverslips

G. Optional: 6-well microplate

Labeling cells with EdU

In initial experiments, we recommend testing a range of EdU concentrations to determine the optimal concentration for your cell type and experimental conditions. If you are currently using a BrdU-based assay for cell proliferation, a similar concentration to BrdU is a good starting concentration for EdU.

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

2.2. Prepare a 2X working solution of EdU in complete medium from the 10 mM stock solution (step 1.2). You can take a final concentration of 10 μM as a start point.(For example, for a final concentration of 10 μM, prepare a 2X working solution of 20 μM).

2.3. 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.**

2.4. 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 depends on the cell growth rate. Pulse labeling of cells by brief exposures to EdU permits studies of cell-cycle kinetics.

2.5. Incubate under conditions optimal for the cell type for the desired length of time. The time of EdU exposure to the cells allows for the direct measurement of cells synthesizing DNA. The choice of time points and length of time for pulsing depends on the cell growth rate.

2.6. Proceed immediately to Cell fixation and permeabilization (steps 3.1 –3.3), followed by EdU detection (steps 4.1–4.7).

Fixation and permeabilization

Transfer the coverslips into a 6-well plate for convenient processing, such that each well contains a single coverslip.

3.1 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.

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

3.3 Remove the wash solution. Add 1 mL of 0.3% Triton® X-100 in PBS to each well, then incubate at room temperature for 10-15 minutes.

Click reaction

4.1 Remove the permeabilization buffer (step 3.3), then wash the cells in each well twice with 1 mL of 3% BSA in PBS. Remove the wash solution

4.2 Prepare 1X EdU buffer additive by diluting the 10X stock solution (prepared in step 1.4) 1:10 in deionized water. Prepare this solution fresh and use the solution on the same day.

4.3 Prepare the Click reaction cocktail. Use this within 15 minutes after preparation.

Reaction components	Number of coverslips			
	1	2	4	5
1X EdU Reaction Buffer	430 µl	860 µl	1.8 ml	2.2 ml
$CuSO_4$ (Component E)	20 µl	40 µl	80 µl	100 µl
6-FAM Azide (Component B)	0.5 µl	1 µl	2 µl	2.5 µl
1X EdU Buffer Additive	50 µl	100 µl	200 µl	250 µl
Total volume	500 µl	1 ml	2 ml	2.5 ml

4.4 Add 0.5 mL of click reaction cocktail to each well containing a coverslip. (You can adjust the volume of cocktail as your preference according to previous experiments.) Rock the plate briefly to insure that the reaction cocktail is distributed evenly over the coverslip.

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

4.6 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.

4.7 Optional: Perform antibody labeling of the samples at this time. It is important to keep the samples protected from light during incubations.

DNA staining

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

5.2 Dilute the Hoechst 33342 (Component G) solution 1:2000 in PBS to obtain a 1X Hoechst 33342 solution (the final concentration is 5 µg/mL).

Note: A range between 2–10 µg/mL of Hoechst 33342 has been shown to work.

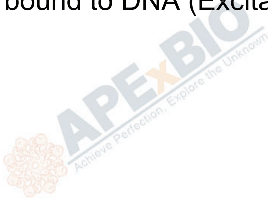
5.3 Add 1 mL of 1X Hoechst 33342 solution per well. Incubate for 15 minutes at room temperature, protected from light. Remove the Hoechst 33342 solution.

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

Imaging and analysis

6-FAM Azide (Excitation maximum: 496 nm Emission maximum: 516 nm)

Hoechst 33342, bound to DNA (Excitation maximum: 350 nm Emission maximum: 461 nm)



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