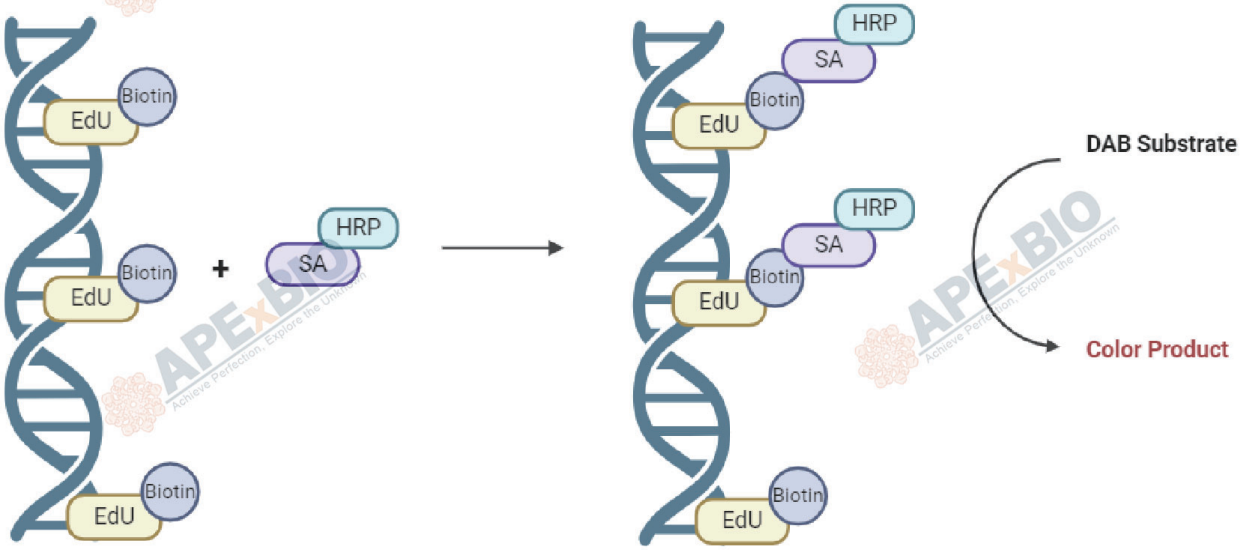
EdU Imaging Kits (DAB)

Introduction

Measuring cell proliferation and cell cycle are a fundamental method to assess cell health, determine genotoxicity, and evaluate drug's pharmacodynamic effect. The common method is measuring DNA synthesis directly. In previous experiments, there are several approaches such as the incorporation of radioactive nucleosides (3H-thymidine) or BrdU. Here, we introduce one new method, click chemistry - CuAAC (Copper-Catalyzed Azide-Alkyne Cycloaddition), and the use of this reaction in direct measurement of S-phase DNA synthesis in cell cycle.

A nucleoside analog of thymidine, EdU (5-ethynyl-2'-deoxyuridine), can be incorporated into DNA strand during DNA synthesis. The alkynyl group of EdU is a biologically inert group that will undergo an extremely selective reaction with dye's azido via a CuAAC reaction to afford an 1,2,3-triazole product. EdU and Biotin azide possess biologically unique moieties to label DNA of proliferating cells, producing low backgrounds and high detection sensitivities. This CuAAC reaction affords superior regioselectivity and quantitative transformation under extremely mild conditions.

EdU Imaging Kits (DAB) specifically labels the DNA of proliferating cells after biotin azide is ligated to EdU, then adds horseradish peroxidase-labeled streptavidin (HRP-Streptavidin) to bind biotin, developing by DAB chromogen, and finally visualized by microscopy.



Components and Storage

Components	50-500 T	200-2000 T
EdU 10mM (Component A)	200 μL	800 μL
Biotin azide (Component B)	55 μL	220 μL
10X EdU Reaction Buffer (Component C)	3.5 mL	13 mL
$CuSO_4$ (100 mM Aqueous Solution) (Component D)	1.2 mL	4.8 mL
10X EdU Buffer Additive (Component E)	1 vial	4 vials
Streptavidin-HRP (Component F)	220 μL	900 μL
Streptavidin-HRP Diluent (Component G)	10 mL	40 mL
DAB Chromogen Solution A (Component H)	10 mL	40 mL
DAB Chromogen Solution B (Component I)	10 mL	40 mL
Store the kit at -20ºC away from moisture, stable for 1 year. Biotin azide and DAB Chromogen Solutions should be stored away from light.		

Protocol

1. Preparing reagents

1) Allow vials to warm to room temperature before opening.

2) Prepare a working solution of 1X EdU reaction buffer (Component C): Dilute Component D in deionized water to make the 1X EdU reaction buffer. After use, store any remaining 1X solution at 2–6ºC. When stored as directed, this 1X solution is stable for up to 6 months.

3) To make a 10X stock solution of the EdU buffer additive (Component E): Add 0.26 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 -20ºC. 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.

2. 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. Endogenous peroxidase blocking solution (for example, 0.3% H_2O_2 in PBS)

E. Deionized water

3. 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.

1) Plate the cells at the desired density, then allow them to recover overnight before additional treatment.

2) Prepare a 2X working solution of EdU in a complete medium from the 10 mM stock solution. 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).

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.

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.

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.

6) Proceed immediately to Cell fixation and permeabilization.

4. Fixation and permeabilization

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) Remove the fixative and wash the cells in each well twice with 1 mL of PBS.

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.

4) Wash the cells in each well twice with 1 mL of PBS.

5) Add 1 mL of 0.3% H_2O_2 PBS per well, and incubate for 10–15 min at room temperature to inactivate endogenous catalase. Subsequently, wash cells 2 times with PBS.

5. Click reaction

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

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

Components	6-well plate			
	1	2	4	5
1X EdU Reaction Buffer	430 μL	860 μL	1.8 mL	2.2 mL
CuSO4 (Component D)	20 μL	40 μL	80 μL	100 μL
Biotin azide (Component B)	1 μL	2 μL	4 μL	5 μL
1X EdU Buffer Additive	50 μL	100 μL	200 μL	250 μL
Total	500 μL	1 mL	2 mL	2.5 mL

3) Add 0.5 mL of click reaction cocktail to each well. (You can adjust the volume of the cocktail to your preference according to previous experiments.) Rock the plate briefly to ensure that the reaction cocktail is distributed evenly.

4) Incubate the plate for 30 minutes at room temperature, protected from light (water can be added to the gaps of the plate during incubation to minimize reaction evaporation).

5) Remove the reaction cocktail, then wash each well once with 1 mL of PBS. Remove the wash solution.

6. DAB staining of samples

1) In between click reaction incubations, prepare the Streptavidin-HRP working solution regarding the table below and mix thoroughly. Streptavidin-HRP working solution must be prepared and used now and cannot be cryopreserved.

Components	6-well plate			
	1	2	4	5
Streptavidin-HRP (Component F)	20 μL	40 μL	80 μL	100 μL
Streptavidin-HRP Diluent (Component G)	180 μL	360 μL	720 μL	900 μL
Total	200 μL	400 μL	800 μL	1 mL

2) Add Streptavidin-HRP working solution and incubate for 30 minutes at room temperature (water can be added to the gaps of the plate during incubation to minimize reaction evaporation).

3) After incubation, wash cells three times with PBS, 3-5 min per time.

4) Mix equal volumes of DAB Chromogen Solution A and DAB Chromogen Solution B to prepare DAB chromogenic solution, add 400 μL of DAB chromogenic solution per well, and incubate for 5-30 min at room temperature. During this period, the color can be observed under the microscope several times, and if the color is dark, it can be 5 minutes.

***Note:** Prepare fresh DAB Chromogen Solution every time.

5) After incubation, wash cells three times with PBS, 3-5 min per time, to stop the color development reaction. If necessary, counterstain with other staining solutions. Observation under an ordinary light microscope or a fluorescence microscope.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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