5-Ethynyl-2'-deoxyuridine (5-EdU)

Introduction

In vivo cell proliferation assays play a crucial role in investigating tumor growth, inflammatory responses, cellular and neural development, hearing and vision impairment, as well as tissue regeneration. Nevertheless, the widespread presence of amines and thiols within cells and culture media complicates the direct labeling of newly synthesized proteins and nucleic acids. Click-iT technology addresses this limitation by utilizing bioorthogonal chemistry with reagents like EdU, which are not naturally found in biological systems, cells, tissues, or model organisms.

EdU can be administered through drinking water, injections (intraperitoneal, intramuscular, or subcutaneous), or direct exposure in specific organisms (e.g., Drosophila and zebrafish larvae), allowing it to be incorporated into actively dividing cells. For initial experiments, it is suggested to test a range of EdU concentrations to identify the optimal dosage. The recommended concentrations can be guided by previous BrdU-based studies. Importantly, lower EdU concentrations often yield fluorescence signals comparable to those obtained with BrdU labeling. The optimal dose varies depending on the labeling duration.

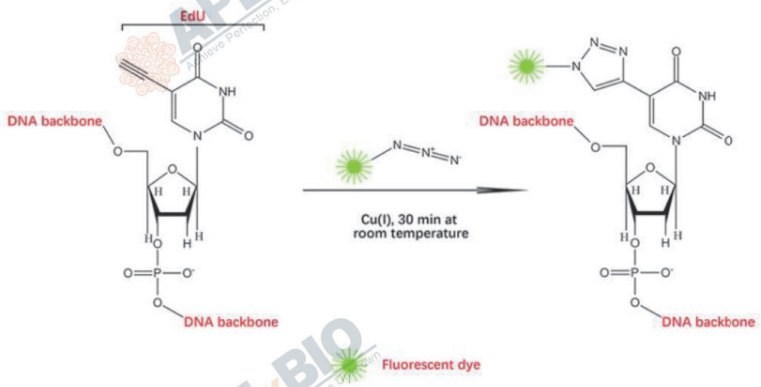

Figure 1. Click reaction(CuAAC) between EdU and azide-modified dye

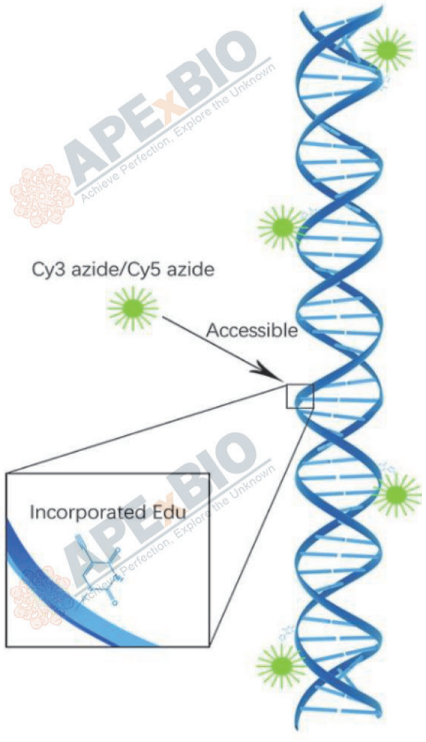

Figure 2. Detection of the incorporated EdU with Cy3/Cy5 azide

Storage

Store desiccated at -20°C, stable for at least 1 year.

Protocol (For in vivo use)

1. Preparation of EdU stock solution

1) Dissolve EdU in sterile saline or PBS at 10 mg/mL and store at -20°C. When stored as directed, the stock solution is stable for up to 1 year.

2. In vivo EdU labeling

1) Injection method: Many injection methods can be used, such as intraperitoneal injection, subcutaneous injection, intramuscular injection and tail vein injection, of which intraperitoneal injection is the most commonly used.

2) Labeling time: The optimal labeling time depends on specific experiment. For fast-proliferating tissues such as the small intestine, labeling time may < 12 h. For slow-proliferating tissues such as the brain, labeling may need a long time (e.g., 7 days or longer).

3) Labeling concentration: The optimal labeling concentration varies, and the recommended concentration is 5-50 mg/kg.

4) Injection time: For short-term labeling (< 24 h), a single injection is enough. For long-term labeling, it may be necessary to use multiple injections.

5) Optical: Some tissue may have non-special dye deposition, it is better to isolate tissue without EdU injection as a negative control.

Table 1. Examples of EdU labeling for in vivo use.

Species	Tissue	EdU Concentration	Administration	Incubation time	Reference
Mice	Primary spongiosa	10 μg/g	Intraperitoneal	4 h	Int. J. Mol. Sci. 2025, 26, 2712.
Mice	Sciatic nerve	50 mg/kg	Intraperitoneal	24 h	J Neurochem. 2025 Jan;169(1):e16281.
Mice	Retinal	50 mg/kg	Intraperitoneal	12 or 24 h	NPJ Regen Med. 2023 Jul 13;8(1):36.
Rat	Brain	50 mg/kg	Intraperitoneal	12 h	Cell Mol Neurobiol. 2023 Aug;43(6):3005-3022.
Rat	Embryonic brain	25 mg/kg	Intraperitoneal (pregnant dam)	-	Front Neuroanat. 2021 Dec 2;15:786329.

3. Preparation of Tissue Sections

1) Sacrifice mice and isolate the tissue of interest. Wash the tissue in PBS several times.

*Note: It is suggested to isolate small intestinal tissue as a positive control. After 6 h of EdU injection in adult mice, the positive signal can be detected.

2) Fix tissue in 4% PFA in PBS, embed in paraffin, section and mount by a standard protocol.

3) Deparaffinize the tissue by a standard protocol like listed below.

Solution	Wash time
Xylene	5 min
Xylene	5 min
100% ethanol	5 min
100% ethanol	3 min
95% ethanol	3 min
85% ethanol	3 min
75% ethanol	3 min
50% ethanol	3 min
PBS	5 min

4) Add 100 µL of 0.3% Triton X-100 in PBS to each slide, then incubate at room temperature for 10-15 min.

5) Wash each slide twice with 100 µL of 0.3% BSA in PBS.

4. EdU detection

Detect EdU with a suitable EdU imaging Kit protocol. Here take EdU imaging Kit (Cy3) (Cat. No: K1075) as an example.

1) Prepare Cy3 Azide working solution: Add 25 µL of DMSO (Component C) to Component B and mix well.

2) Prepare 1X EdU reaction buffer: Dilute 10X EdU Reaction Buffer (Component D) in deionized water to make the 1X EdU reaction buffer.

3) Prepare 10X stock solution of the EdU buffer additive: Add 2 mL of deionized water to the vial of EdU Buffer Additive (Component F) and mix until the EdU buffer additive is fully dissolved. If the solution develops a brown color, it has degraded and should be discarded.

4) 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.

5) Prepare the Click reaction cocktail. Use this within 15 min after preparation.

Components	Slides			
	5	10	20	25
1X EdU Reaction Buffer	430 µL	860 µL	1.8 mL	2.2 mL
CuSO4 (Component E)	20 µL	40 µL	80 µL	100 µL
Cy3 azide	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

6) Add 100 μL of click reaction cocktail to each slide. (You can adjust the volume of the cocktail to your preference according to previous experiments.) Make sure that the reaction cocktail is distributed evenly.

7) Incubate for 30 min at room temperature, protected from light.

8) Remove the reaction cocktail, then wash the slide once with 100 μL of 0.3% BSA in PBS. Remove the wash solution.

9) Optional: Wash cells twice with methanol, 5 min per time. Wash cells twice with PBS, 5 min per time.

***Note:** Some tissue may have non-special dye deposition, this step can reduce the background signal.

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

5. DNA staining

1) Wash each slide with 100 μL of PBS. Remove the wash solution.

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).

3) Add 100 μL of 1X Hoechst 33342 solution to each slide. Incubate for 15 min at room temperature, protected from light. Remove the Hoechst 33342 solution.

4) Wash each slide twice with 100 μL of PBS. Remove the wash solution and mount with a standard protocol.

6. Imaging

1) Proceed to detection in a fluorescence with filters for Cy3 (Ex/Em: 555/570 nm) and Hoechst 33342 (Ex/Em: 350/461 nm).

Note

1. This product needs to be used in conjunction with EdU imaging Kit or EdU Flow Cytometry Assay Kits.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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