**EdU Cell Proliferation Assay Kit (Fluorescein 488,Green)**

**Catalog No.: RA20069**

## Basic Information

| | |
|---|---|
| **Product name** | EdU Cell Proliferation Assay Kit (Fluorescein 488,Green) |
| **Sizes** | 20T/100T/500T |
| **Storage** | -20℃, keep away from light |
| **Shipping** | Shipped with ice pack |
| **Validity** | 12 months |
| **Ex/Em** | 495/519 nm |

## Product Introduction

Cell proliferation detection is a fundamental experimental method for assessing cell health, genetic toxicity, and the efficacy of anti-tumor drugs. BrdU is a conventional method for cell proliferation labeling. EdU improves upon BrdU with simpler operation and higher detection sensitivity. EdU (5-ethynyl-2'-deoxyuridine) is a pyrimidine analog that integrates into DNA during the DNA synthesis phase. The detection is based on a "click" reaction, a covalent reaction catalyzed by copper between azide compounds and alkynes. The EdU labeling method is rapid and efficient, easy to use. It only requires formaldehyde fixation and Triton X-100 permeabilization to allow the detection reagent to enter the cells. A small amount of azide dye can effectively label the integrated EdU. In contrast, the BrdU method requires DNA denaturation (such as acid denaturation, heat denaturation, or DNase digestion) to expose BrdU for antibody binding. This kit includes all the components required for EdU detection and can be used for the proliferation detection of in vitro cultured cells.

## Application Scope

Cell proliferation, differentiation, growth and development, DNA damage repair, viral replication and other aspects of research.

## Product features

**Efficient and Simple:** This detection method, based on small molecule chemistry, eliminates antigen-antibody reactions and requires only a few minutes.

**High Sensitivity:** Using BrdU as the detection dye at 1/500th concentration of conventional antibodies, it achieves high sensitivity with easy diffusion, enabling accurate detection even in single proliferating cells.

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**Time-Saving:** The process eliminates overnight incubation and complex antigen-antibody reactions, completing the entire detection cycle in just 5 hours.

## Product Components

| components | 2~20 T | 10~100 T | 50~500 T | Storage Temperature After Opening | stability |
|---|---|---|---|---|---|
| A. 10 mM EdU | 40 µL | 200 µL | 1 mL | -20 ℃ | After opening, different components are stored at the specified temperature, such as subsequent storage-20℃, which is also possible. |
| B. 488A Azide | 10 µL | 50 µL | 250 µL | -20 ℃, protect form light | |
| C. 10 × Click-iT EdU reaction buffer | 200 µL | 1 mL | 5 mL | 2~8 ℃ | |
| D. $CuSO_4$ | 100 µL | 500 µL | 2 × 1.25 mL | 2~8 ℃ | |
| E. Click-iT EdU Buffer additives | 6 mg | 30 mg | 150 mg | 2~8 ℃ | |
| F. Hoechst 33342 | 5 µL | 25 µL | 125 µL | 2~8 ℃ | |

**Specifications: For fluorescence microscopy applications, the maximum number of uses per kit is the highest specified for the 96-well plate culture system, such as the 20T kit which can process 20-well samples (specific volumes for different containers are referenced in Table 2). For flow cytometry applications, the minimum number of uses per kit is the lowest specified for the same system, with the 20T kit designed to process 2 samples.**

## Materials Required (Not Supplied)

1.Consumables: 96/24/12/6 culture plates or petri dishes

2.Reagents:

(1) 10 mM PBS (pH 7.2-7.6)

(2) 4% polyformin fixative (in PBS)

(3) Osmotic enhancer (0.5% Triton X-100 in PBS)

(4) 2 mg/mL glycine solution (in ddH2O)

(5) 3% BSA in PBS (pH 7.2-7.6)

(6) 1% BSA in PBS (pH 7.2-7.6)

(7) ddH2O

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## Perimental procedure

1.Fluorescence Microscopy Detection Method

(1) Cell Culture:

Take logarithmic growth phase cells and inoculate them into 96-well plates at a concentration of 4 × $10^3$ to 1 × $10^5$ cells per well (adjust cell quantity and density according to cell size, growth rate, and experimental requirements). Cultivate until reaching normal growth stage.

(2)Drug Treatment:

Perform various drug treatments as required for the experiment.

(3)EdU Labeling

1) Dilute EdU solution (component A) in complete cell culture medium to appropriate concentration using a specific ratio, then add to cells and mix thoroughly. Establish a negative control group without EdU treatment.

**Note: Adjust EdU labeling concentration based on cell type. It is recommended to start with an initial concentration of 10 μM for optimization. For preliminary experiments, set up an EdU concentration gradient as referenced in Tables 3 and 4.**

2)Incubate in a cell incubator for 2 hours.

**Note: Optimal incubation time depends on the cell cycle. Most tumor cell lines can tolerate 2-hour incubation (see Table 3). EdU concentration should be adjusted according to incubation duration: higher concentrations (e.g., 10-50 μM) are recommended for short-term incubation (<2 h), while lower concentrations (e.g., 1-10 μM) are preferred for extended incubation (> 24 h).**

(4)Cell Fixation and Osmotic Pressure Enhancement:

For experiments requiring surface antigen labeling, wash cells twice with 3% BSA buffer after EdU incubation before performing fixation and osmotic pressure enhancement.

1)After incubation, remove the culture medium. Wash cells twice with 1× PBS for 5 minutes each time to eliminate residual EdU not incorporated with DNA. Reduce washing intensity for cells with loose adhesion. Add 50 μL 4% polyformaldehyde fixative and incubate at room temperature for 20 minutes before removing the fixative.

2) Add 50 μL 2 mg/mL glycine solution per well and incubate at room temperature for 5 minutes to neutralize residual fixative.

3) Wash cells twice with 100 μL 3% BSA per well.

4) Remove wash buffer and add 100 μL 0.5% Triton X-100, incubating at room temperature for 10 minutes.

(5) EdU Detection Note: This reference protocol uses 100 μL working solution per sample. Adjust the volume according to your sample conditions.

1) Prepare 1× Click-iT EdU reaction buffer (Component C): Dilute Component C with ddH2O 10 times.
2) Prepare 5× Click-iT EdU buffer additive (Component E): Add 300 μL ddH2O to 30 mg of Component E tube (final concentration 100 mg/mL), mix thoroughly. Store remaining buffer at-20℃ for one year. If the solution turns brown, it indicates degradation of active components and inactivation.

**Note: Components E of different specifications are dissolved in ddH2O at the specified ratios to prepare 5× stock solutions for later use.**

3)Prepare 1× Click-iT EdU buffer additive: Dilute the ddH2O to 1× Click-iT EdU buffer additive. The solution should be prepared and used immediately.

4) Prepare Click-iT working solution according to Table 1.

Table 1 Click-iT working fluid

| Reaction components | Take a sample of 10 holes |
|---|---|
| 1 × Click-iT EdU reaction buffer | 855 μL |
| CuSO4 (component D) | 40 μL |
| 488A Azide (component B) | 5 μL |
| 1 × Click-iT EdU Buffer additives | 100 μL |
| Total volume | 1 mL |

5)Remove the osmotic agent. Wash each well twice with 100 μL of 3% BSA buffer.
6) Add 100 μL Click-iT working solution per well, ensuring uniform coverage of cells.
7) Incubate at room temperature in the dark for 30 minutes. 8) Discard the Click-iT working solution. Wash cells twice with 100 μL of 3% BSA buffer, remove the washing liquid, and add 100 μL PBS to maintain cell moisture. If no special requirements are specified, proceed to image analysis.

(6) DNA Re-labelling (optional)

1) Wash cells once with 100 μL PBS and discard the washing liquid.
2) Dilute Hoechst 33342 (Component F) in PBS at a 2000-fold concentration.
3) Add 100 μL of 1× Hoechst 33342 solution per well, incubate at room temperature in the dark for 15-30 minutes.
4) Discard the Hoechst 33342 solution and wash cells twice with 100 μL PBS.

(7) Imaging and Analysis It is recommended to perform fluorescence microscopy imaging immediately after staining. If conditions are limited, complete imaging within 3 days under 4°C light-shielding moist storage conditions.

2. Flow Cytometry Detection Method
(1) Cell Culture: 1×10^5-3×10^6 cells per well are inoculated into a 6-well plate.
(2) Drug Treatment: Various drugs are administered according to experimental requirements.
(3) EdU-labeled Cells
1) Add the diluted EdU solution (Component A) to the cell culture medium at appropriate concentrations, mix thoroughly, and establish a negative control group without EdU. **Note: The EdU concentration should be adjusted based on cell type, with an initial concentration of 10 μM recommended for optimization. For preliminary experiments, an EdU concentration gradient should be established using reference values from Tables 3 and 4.**
2) Incubation in a cell culture incubator: 2 hours. The EdU incubation duration directly serves as an indicator for DNA synthesis measurement, with timing and incubation duration determined by cell growth rates. Pulsed EdU labeling can be used to study cell cycle dynamics. Note: Optimal incubation time depends on the cell cycle phase, with most tumor cell lines suitable for 2-hour incubation (see Table
3). EdU concentration should be adjusted according to incubation duration: high concentrations (e.g., 10-50 μM) are recommended for short-term incubations (<2 h), while low concentrations (e.g., 1-10 μ M) are preferred for extended incubations (> 24 h); reference values from Table 4 may also apply.
(4) Cell Fixation and Osmotic Pressure Enhancement: For experiments requiring cell surface antigen labeling, consider washing cells twice with 1% BSA after EdU incubation before proceeding to fixation and osmotic pressure enhancement.
1) After incubation, collect cells and add 1 mL PBS per tube for washing. Centrifuge at 1000 rpm for 5 minutes, then aspirate the supernatant to remove residual EdU without DNA incorporation.
2) Resuspend cells in 1 mL 4% polyformaldehyde fixative per tube.
3) Incubate at room temperature for 20 minutes, then centrifuge at 1000 rpm for 5 minutes. Aspirate the supernatant.
4) Incubate with 1 mL 2 mg/mL glycine for 5 minutes to neutralize residual fixative. Centrifuge at 1000 rpm for 5 minutes, then aspirate the supernatant. Wash each tube once with 1 mL PBS at 1000 rpm for 5 minutes, followed by another aspirate.
5) Resuspend cells in 1 mL 0.5% Triton X-100 osmotic pressure enhancer and incubate at room temperature for 10 minutes.
(5) EdU Detection: For 6-well plate samples, use 1 mL working solution per well as recommended. Users may adjust concentration based on sample characteristics.
1) Prepare 1× Click-iT EdU reaction buffer: Dilute component C with ddH2O to 1:10 ratio.
2) Preparation: Add 300 μL ddH2O to the 30 mg component E test tube (final concentration 100

mg/mL) and mix thoroughly until completely dissolved. The remaining stock solution should be stored at-20℃°C for one year. If the solution turns brown, it indicates degradation of active components and rendering it unusable. Note: All component E stock solutions of different specifications should be diluted with ddH2O in this ratio to prepare 5× stock solutions for later use.

3) Preparation: Dilute the 5× Click-iT EdU buffer additive stock solution with ddH2O to 1× volume. The prepared solution should be used immediately.

4) Prepare Click-iT working solution according to Table 2.

Table 2 Click-iT working fluid

| Reaction components | Volume of liquid required for a single reaction |
|---|---|
| 1 × Click-iT EdU reaction buffer | 875 μL |
| CuSO4（component D） | 20 μL |
| 488A Azide（component B） | 5 μL |
| 1 × Click-iT EdU Buffer additives | 100 μL |
| Total volume | 1 mL |

5)Spin at 1000 rpm for 5 minutes. Aspirate the supernatant and remove the osmotic agent. Add 1mL of 1% BSA wash buffer to each tube, wash twice, then spin at 1000 rpm for 5 minutes. Aspirate the supernatant.

6) Add 1 mL of Click-iT working solution to each tube and mix thoroughly.

7) Incubate at room temperature in the dark for 30 minutes.

8) Spin at 1000 rpm for 5 minutes. Aspirate the staining reaction solution, add 1% BSA to each tube twice for washing cells, then spin at 1000 rpm for 5 minutes. Aspirate the supernatant and resuspend cells in 1 mL of 1% BSA (adjust the volume of the resuspended cell suspension according to cell quantity). Perform flow cytometry analysis. Note: For additional marker detection, refer to step 4.

(6) DNA Re-labelling

1) Wash cells once with 100 μL PBS and remove the wash solution.

2) Dilute Hoechst 33342 (component F) with PBS at a 2000-fold dilution.

3) Add 100 μL of 1× Hoechst 33342 solution per well and incubate at room temperature in the dark for 15-30 minutes.

4) Remove the Hoechst 33342 solution and wash cells twice with 100 μL PBS.

(7) Intracellular antigen labeling (optional)

1) Add antibody working solution and mix thoroughly.

2) Incubate the antibody under light-free conditions at an appropriate temperature and duration.

(8) Flow Cytometry Analysis

1) It is recommended to perform flow cytometry immediately after staining. If conditions do not permit, store samples in a dark 4℃ moist environment for up to 3 days.

2) The cell count should ideally reach the million-level. For smaller cell counts, start with 100,000 cells for analysis. If cell yield is too low (just reaching the 10,000 level), which may compromise flow cytometry results, reduce the number of washing cycles specified in Section (V) 8.

Table 3 Reference usage of EdU culture medium and staining reaction solution

| Reagent | 96-well plate | 48-well plate | 24-well plate | 12-well plate | 6-well plate | 5.5 cm small plate |
|---|---|---|---|---|---|---|
| EdU Medium | 100 µL | 150 µL | 200 µL | 500 µL | 1 mL | 2 mL |
| Staining solution | 100 µL | 150 µL | 200 µL | 500 µL | 1 mL | 2 mL |

Table 4 Reference incubation time for EdU

| clone | human embryonic cells | yeast cell | Mouse fibroblasts | human cervical carcinoma cell | Human embryonic renal cell lines | Human nerve cells |
|---|---|---|---|---|---|---|
| cell cycle | ~30 min | ~3 h | ~18 h | ~21 h | ~25 h | ~5 d |
| incubating time | 5 min | 20 min | 2 h | 2 h | 2 h | 1 d |

**Note: 1. EdU incubation time depends on the cell cycle, generally 1/10 to 1/5 of the cell cycle, but most cell lines can be incubated for 2 h.**

**2.The cell cycle may vary due to other factors such as cell culture medium, temperature, humidity, light, etc.**

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## Notes

1.Before use, centrifuge the product to the bottom of the tube immediately before proceeding with subsequent experiments.

2. EdU (10 mM) should be aliquoted according to experimental requirements for initial use and stored at-20°C.

3. This product should not be tested simultaneously with TUNEL reagent kits, as the-OH groups in EdU's structure may interfere with TUNEL's reaction process.

4. Since copper ions can disrupt actin structure and affect digoxin detection, this product is incompatible with Phalloidin (digoxin).

5. After opening, store components according to the instructions provided in the package.

6. Fluorescent dyes are prone to quenching; ensure light protection during operation to minimize fluorescence quenching.

7. For safety, wear lab coats and disposable gloves during handling.

8. This product is for research purposes only and must not be used for clinical diagnosis or treatment.

**This product is for research use only!**