# Elabscience® E-Click EdU Cell Proliferation Flow Cytometry Assay Kit (Green, FITC)

Catalog No: E-CK-A370

Product size: 50 Assays/200 Assays

## Components

| Cat. | Products | 50 Assays | 200 Assays | Storage |
|---|---|---|---|---|
| E-CK-A37A | EdU(10mM) | 200 μL | 800 μL | -20℃/-80℃, shading light |
| E-CK-A37B1 | Click Reaction Buffer I | 25 mL | 50 mL×2 | -20°C |
| E-CK-A370C | FITC Azide I | 60 μL | 250 μL | -20℃/-80℃, shading light |
| E-CK-A37D | $CuSO_4$ | 1.25 mL×2 | 8 mL | -20°C |
| E-CK-A37E | Click Additive | 220 mg | 220 mg×4 | -20°C |
| Manual | One Copy | | | |

Note: 50 Assays means that 50 samples can be detected in 6-well plate.

## Introduction

Elabscience® E-Click EdU Cell Proliferation Flow Cytometry Assay Kit is easy to operate and has high sensitivity. It is suitable for the proliferation assay of suspension cells, and the results can be analyzed by flow cytometry.

## Detection Principle

Cell proliferation assays are widely used in the evaluation of cell viability, genotoxicity, and the effect of antitumor drugs. Direct detection of DNA synthesis in cells is considered to be the most accurate method for detecting cell proliferation. The initial widely used method for detecting DNA synthesis in cells was the radiolabeled nucleoside incorporation method, but this method was greatly limited due to radioactive contamination and the difficulty of single-cell detection, and was gradually replaced by the BrdU method based on antibody detection. The BrdU method has many steps and requires the use of BrdU antibody, which has many influencing factors and poor stability.

EdU method is based on EdU incorporation and subsequent click reaction, without the use of antibodies, convenient operation and high detection sensitivity. It is a new method upgraded on the basis of BrdU method and will gradually replace BrdU method.

EdU (5-ethynyl-2-deoxyuridine), is a thymidine analog, EdU can replace thymidine in the process of DNA synthesis to incorporate into new in synthetic DNA. On the other hand, the acetylene group on EdU can react with fluorescently labeled small molecule azide probes (such as FITC Azide, Elab Fluor® 488 Azide, Elab Fluor® 594 Azide, Elab Fluor® 647 Azide) through the catalysis of monovalent copper ions to form a stable triazole ring. This reaction is very rapid and is called the click reaction. Through the click reaction, the newly synthesized DNA is labeled with the corresponding fluorescent probe, so that the proliferating cells can be detected using the appropriate fluorescent detection equipment.

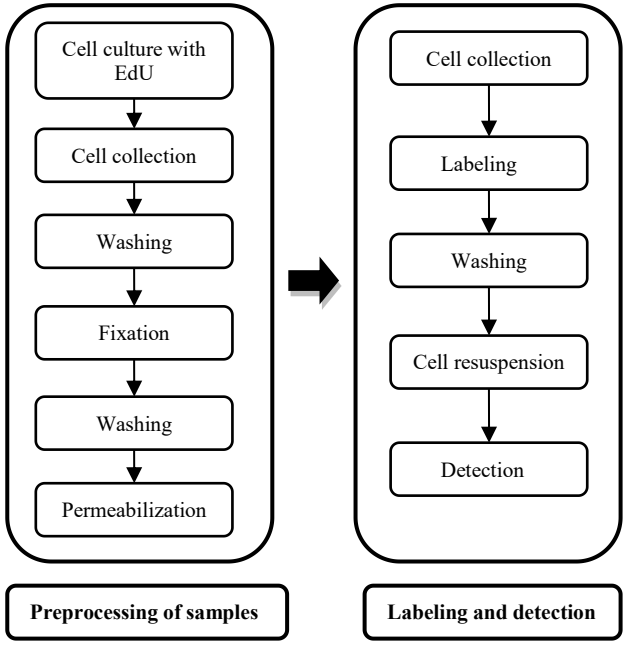
## Detection Sample Types

☑ **Suspension cells** ☑ **Adherent cells**

## Storage

Store at -20°C for 12 months. EdU (10 mM) needs to be stored in aliquots (50 μL/vial is recommended or aliquot into smaller quantities according to experiental needs) for the first use.

## Assay Procedure




## Materials Not Supplied

**1) Reagents**

PBS (with 1% BSA) (pH7.2~7.6).

Permeabilization buffer: 1% Saponin (dissolved in PBS, pH7.2~7.6).

Fixation buffer: 4% Polyformaldehyde (dissolved in PBS).

Deionized water.

**2) Instrument**

Flow cytometry, centrifuge.

## Reagent Preparation

**1) Click Additive Solution:**

Dissolve a vial of Click Additive (220 mg) with 1.1 mL deionized water fully. Aliquot the prepared solution and store at -20ºC. (It is recommended to open a new vial of Click Additive after using one tube).

## Experimental Operation

**1. Cell culture with EdU**

1) The labeling concentration of EdU varies with different cell types. Cell culture medium, cell growth density, cell type and other experimental conditions may affect the labeling effect of EdU. Therefore, the labeling concentration of EdU needs to be confirmed by preliminary experiments. It is recommended to use the initial concentration of 10 μM to performe the preliminary experiment.
2) In preliminary experiments, it is recommended to set up different concentration gradients of EdU staining solution to determine the best concentration. Table 2. *EdU Incubation Time for Common Cell Lines* and table 3. *Reference for EdU Incubation Concentration and Time in Cell Experiments* can be used as reference.
Note: It is recommended to use cell sample without EdU as a negative.

**2. Fixation and Permeabilization**

*The volume of reagents used in the following steps is suitable for 6-well plate. For other microplate, it can be adjusted appropriately according to experimental needs.*

1) Collect the cells, centrifuge at 300×g for 5 min, discard the supernatant.

2) Wash the cells with 1mL of PBS (with 1% BSA), then centrifuge at 300×g for 5 min, discard the supernatant.
3) Resuspend the cells with 1mL4% Polyformaldehyde (dissolved in PBS) and mix fully, then incubate the cells at RT for 15 min with shading light.
4) Centrifuge at 300×g for 5 min, discard the supernatant, then resuspend the cells with 1mL PBS (with 1% BSA) and mix fully.
5) Centrifuge at 300×g for 5 min, discard the supernatant, then resuspend the cells with 1mL PBS (with 1% BSA) and mix fully.
6) Centrifuge at 300×g for 5 min, discard the supernatant, then resuspend the cells with 0.5mL PBS (with 1% Saponin) and mix fully, incubate the cells at RT for 20 min.

### 3. Labeling

**This manual is based on the total reaction volume of 500 μL per well of 6-well plate. For other types of well plates, the volume of Click Reaction Solution added to each well refers to Appendix Table 1.**

1) According to the number of samples, refer to the following table to prepare Click Reaction Solution.

| Ingredient | Sample size | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 5 | 10 | 25 | 50 |
| Click Reaction Buffer I | 440 μL | 880 μL | 1.76 mL | 2.2 mL | 4.4 mL | 11 mL | 22 mL |
| $CuSO_4$ | 40 μL | 80 μL | 160 μL | 200 μL | 400 μL | 1 mL | 2 mL |
| FITC Azide I | 1 μL | 2 μL | 4 μL | 5 μL | 10 μL | 25 μL | 50 μL |
| Click Additive Solution | 20 μL | 40 μL | 80 μL | 100 μL | 200 μL | 500 μL | 1 mL |
| Total volume | 500 μL | 1 mL | 2 mL | 2.5 mL | 5 mL | 12.5 mL | 25 mL |

Note:
a) Please strictly prepare the Click Reaction Solution in accordance with the order and volume of the ingredients in the above table, otherwise it will affect the result.
b) Click Reaction Solution should be used within 15 min after preparation.

2) Centrifuge at 300×g for 5 min, discard the supernatant, then add 500 μL of Click Reaction Solution and mix fully, incubate the cells at RT for 30 min.
3) Centrifuge at 300×g for 5 min, discard the supernatant, then resuspend the cells with PBS (with 1% Saponin) and mix fully.
4) Centrifuge at 300×g for 5 min, discard the supernatant, add 200 μL of PBS (with 1% BSA) to resuspend the cells, and detect by flow cytometry.

Note:
a) The maximum excitation wavelength of FITC is 490 nm and the maximum emission wavelength is 530 nm.
b) Please detect as soon as possible to avoid fluorescence quenching.

## Appendix

**Table 1 Usage of Click Reaction Solution**

| | 96-well plate | 48-well plate | 24-well plate | 12-well plate | 6-well plate |
|---|---|---|---|---|---|
| Click Reaction Solution | 100 μL | 150 μL | 250 μL | 400 μL | 500 μL |

**Table 2 Incubation time of EdU for Common cells**

| Cell type | Human embryonic cells | Yeast cells | 3T3 | Hela | HEK293 | Human nerve cells |
|---|---|---|---|---|---|---|
| Doubling time | ~30 min | ~3 h | ~18 h | ~21 h | ~25 h | ~5 d |
| Incubation time | 5 min | 20 min | 2 h | 2 h | 2 h | 1 d |

**Table 3 the reference of Incubation concentration and time of EdU**

| PubMed ID | Reference | Cell line | Concentration | Time |
|---|---|---|---|---|
| 19647746 | Yu Y, et al. J Immunol Methods. 2009 | Spleen cells | 50 μM | 24 h |
| 19544417 | Momcilović O, et al. Stem Cells. 2009 | Human ES cells | 10 μM | 0.5 h |
| 20080700 | Cinquin O, et al. PNAS. 2010 | emb-30 | 1 μM | 12 h |
| 20025889 | Han W, et al. Life Sci. 2009 | VSMC | 50 μM | 2 h |
| 20659708 | Huang C, et al. J Genet Genomics. 2010 | ESC | 50 μM | 2 h |
| 21310713 | Hua H, et al. Nucleic Acids Res. 2011 | Fission yeast strains | 10 μM | 3 h |
| 20824490 | Lv L, et al. Mol Cell Biochem. 2011 | EJ cells | 50 μM | 4 h |
| 21248284 | Yang S, et al. Biol Reprod. 2011 | GC cells | 50 μM | 2 h |
| 21227924 | Zhang YW, et al. Nucleic Acids Res. 2011 | U2OS, HT29 | 30 μM | 1.5 h |
| 21829621 | Guo T, et al. PloS One. 2011 | HIT-T15 | 50 μM | 4 h |
| 21980430 | Zeng T, et al. PloS One. 2011 | MCF-10A | 25 μM | 2 h |
| 22012572 | Ding D, et al. Int Orthop. 2011 | C3H10T1/2 | 10 μM | 24 h |
| 22000787 | Zeng W, et al. Biomaterials. 2011 | EPC | 50 μM | 4 h |
| 21913215 | Xue Z, et al. J Cell Biochem. 2011 | SGC7901 | 25 μM | 24 h |
| 22016038 | Peng F, et al. Lasers Med Sci. 2011 | MSC | 50 μM | 2 h |
| 21878637 | Li D, et al. J Biol Chem. 2011 | HCC | 50 μM | 2 h |

## Declaration

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. The labeling concentration of EdU should be optimized according to the cell type used. It is recommended to do a preliminary experiment to explore the optimal concentration of EdU and 10 μM EdU can be used as initial exploratory concentration.
4. Since the EdU labeling reaction is carried out in the cells and detected by flow cytometry, please ensure that the cells are completely fixed and permeabilized before EdU labeling. If the room temperature is too low such as in winter, it is recommended to extend the fixation time appropriately or fix it overnight at 4°C.
5. Aliquot the Click Additive Solution and store at -20ºC. If white substance is precipitated before use, please turn it upside down several times and use it only after it has completely dissolved. If the color of the Click Additive Solution turns brown, indicates that the reagent has expired, please discard it.
6. Copper ions will affect the fluorescence of GFP, RFP, mCherry and other fluorescent proteins, so this kit is not suitable for cells with GFP, RFP, mCherry and other fluorescence.