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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Elabscience[®] E-Click EdU Cell Proliferation Flow Cytometry Assay Kit (Green, Elab Fluor[®] 488)

Catalog No: E-CK-A371

Product size: 50 Assays/200 Assays

This manual must be read attentively and completely before using this product.
If you have any problems, please contact our Technical Service Center for help (info
in the header of each page).

Tel: 240-252-7368(USA)

Fax: 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit
for more efficient service.

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

Components

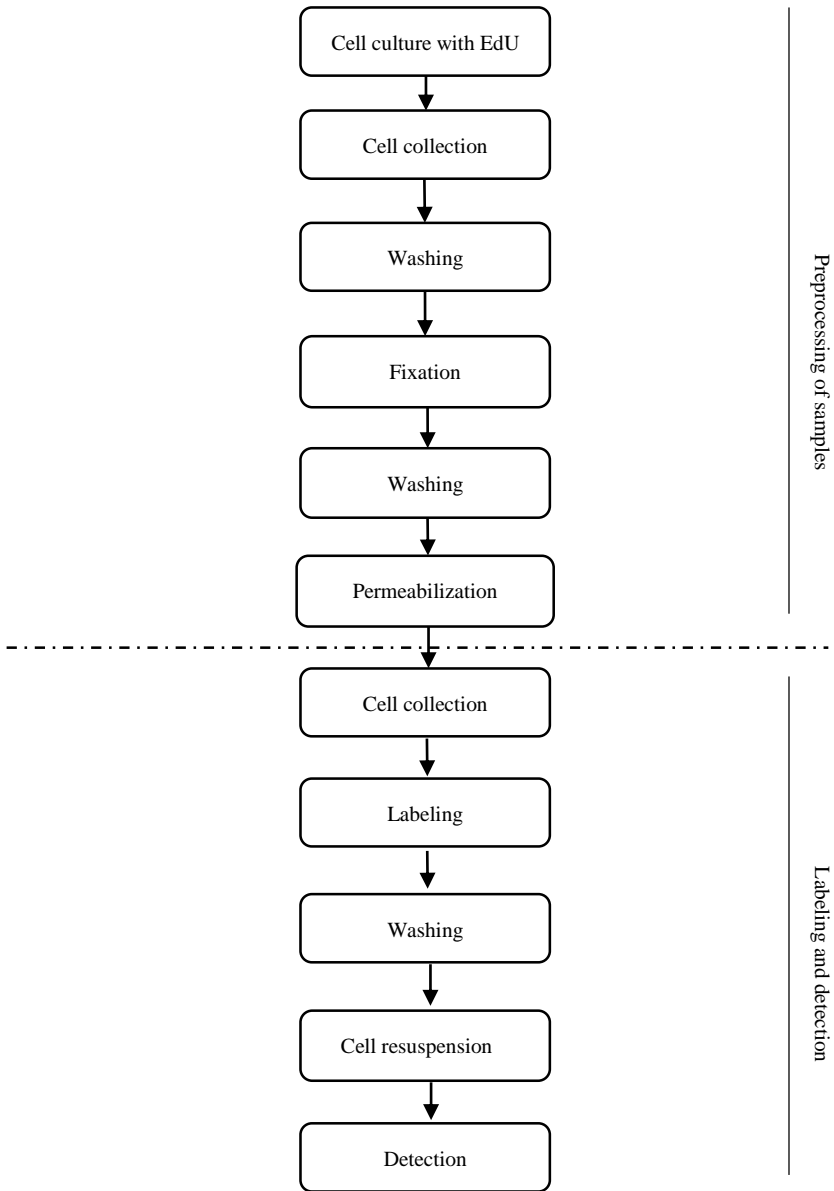
Cat	Products	50 Assays	200 Assays	Storage
E-CK-A37A	EdU(10mM)	200 μ L	800 μ L	-20 $^{\circ}$ C
E-CK-A37B1	Click Reaction Buffer I	25 mL	50 mL \times 2	-20 $^{\circ}$ C
E-CK-A371C	Elab Fluor [®] 488 Azide I	50 μ L	200 μ L	-20 $^{\circ}$ C, shading light
E-CK-A37D	CuSO ₄	1.25 mL \times 2	8 mL	-20 $^{\circ}$ C
E-CK-A37E	Click Additive	200 mg	200 mg \times 4	-20 $^{\circ}$ C
Manual	One Copy			

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

Storage

Store at -20 $^{\circ}$ C for 12 months. EdU (10 mM) needs to be stored in aliquots (50 μ L/vial is recommended or aliquot into smaller quantities according to experiential 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

Click Additive Solution:

Dissolve a vial of Click Additive (200 mg) with 1 mL deionized water fully.

Aliquot the prepared solution and store at -20 °C. (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 μ M to perform 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 1mL 4% 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 μ L 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 μ L	880 μ L	1.76 mL	2.2 mL	4.4 mL	11 mL	22 mL
CuSO ₄	40 μ L	80 μ L	160 μ L	200 μ L	400 μ L	1 mL	2 mL
Elab Fluor [®] 488 Azide I	1 μ L	2 μ L	4 μ L	5 μ L	10 μ L	25 μ L	50 μ L
Click Additive Solution	20 μ L	40 μ L	80 μ L	100 μ L	200 μ L	500 μ L	1 mL
Total volume	500 μ L	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 \times g for 5 min, discard the supernatant, then add 500 μ L of Click Reaction Solution and mix fully, incubate the cells at RT for 30 min.
 - 3) Centrifuge at 300 \times g for 5 min, discard the supernatant, then resuspend the cells with PBS (with 1% Saponin) and mix fully.
 - 4) Centrifuge at 300 \times g for 5 min, discard the supernatant, add 200 μ L of PBS (with 1% BSA) to resuspend the cells, and detect by flow cytometry.

Note:

- a) The maximum excitation wavelength of Elab Fluor[®] 488 is 495 nm and the maximum emission wavelength is 519 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 μ L	150 μ L	250 μ L	400 μ L	500 μ L

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 μ M	24 h
19544417	Momcilović O, et al. Stem Cells. 2009	Human ES cells	10 μ M	0.5 h
20080700	Cinquin O, et al. PNAS. 2010	emb-30	1 μ M	12 h
20025889	Han W, et al. Life Sci. 2009	VSMC	50 μ M	2 h
20659708	Huang C, et al. J Genet Genomics. 2010	ESC	50 μ M	2 h
21310713	Hua H, et al. Nucleic Acids Res. 2011	Fission yeast strains	10 μ M	3 h
20824490	Lv L, et al. Mol Cell Biochem. 2011	EJ cells	50 μ M	4 h
21248284	Yang S, et al. Biol Reprod. 2011	GC cells	50 μ M	2 h
21227924	Zhang YW, et al. Nucleic Acids Res. 2011	U2OS, HT29	30 μ M	1.5 h
21829621	Guo T, et al. PloS One. 2011	HIT-T15	50 μ M	4 h
21980430	Zeng T, et al. PloS One. 2011	MCF-10A	25 μ M	2 h
22012572	Ding D, et al. Int Orthop. 2011	C3H10T1/2	10 μ M	24 h
22000787	Zeng W, et al. Biomaterials. 2011	EPC	50 μ M	4 h
21913215	Xue Z, et al. J Cell Biochem. 2011	SGC7901	25 μ M	24 h
22016038	Peng F, et al. Lasers Med Sci. 2011	MSC	50 μ M	2 h
21878637	Li D, et al. J Biol Chem. 2011	HCC	50 μ M	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 $^{\circ}\text{C}$.
5. Aliquot the Click Additive Solution and store at -20 $^{\circ}\text{C}$. 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.