

IK-873 IS-Click EdU Cell Proliferation Imaging Assay Kit (RED- Alexa Fluor 647)

Size: 50 Assays/200 Assays

Introduction

IS-Click EdU Cell Proliferation Imaging Assay Kit is easy to operate and has high sensitivity. It is suitable for the proliferation assay of cell slides and smears, and the results can be observed by flow fluorescence microscope.

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, Alexa Fluor 488 Azide, Alexa Fluor 594 Azide, Alexa 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

☒ Cell Slides ☒ Cell Smears

Cat.	Products	IK-873 -50 50 Assays	IK-873-200 200 Assays	Storage
IS-873A	EdU(10mM)	200 μ L	800 μ L	-20°C
IS-873B2	Click Reaction Buffer II	25 mL	50 mL×2	-20°C
IS-873C	Alexa Fluor 647 Azide	60 μ L	250 μ L	-20°C, shading light
IS-873D	CuSO ₄	1.25 mL×2	8 mL	-20°C
IS-873E	Click Additive	220 mg	220 mg×4	-20°C
IS-873	DAPI Reagent(25 μ g/mL)	1.25 mL	1.25 mL×4	-20°C, shading light
Manual	One Copy			

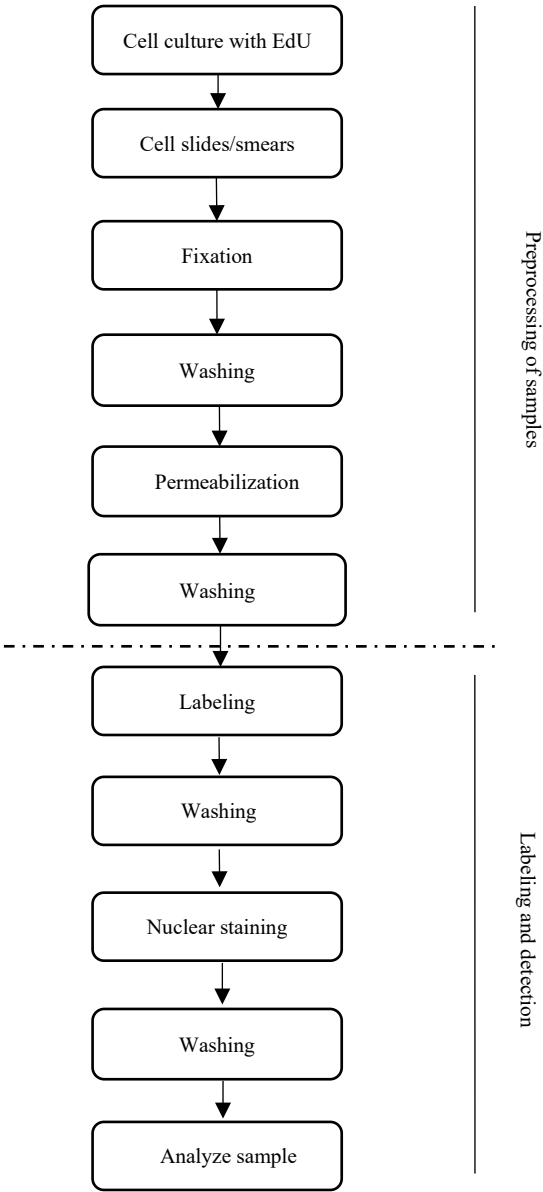
Components

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

Storage

Store at -20°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 (pH7.2~7.6).

PBS (with 3% BSA) (pH7.2~7.6).

Permeabilization buffer: 0.3% Triton X-100 (dissolved in PBS, pH7.2~7.6).

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

Deionized water.

2) Instrument

Fluorescence microscope.

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 -20°C. (It is recommended to open a new vial of Click Additive after using one tube).

2) DAPI working solution:

Add 4 µL DAPI Reagent (25µg/mL) to 96 µL PBS and mix well. Prepare the fresh solution before use.

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 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) After incubation, discard the medium.
- 2) Add 1mL of 4% Polyformaldehyde (dissolved in PBS) to each well, incubate at RT for 15 min, and then remove the 4% Polyformaldehyde (dissolved in PBS).
- 3) Add 1mL of PBS (with 3% BSA) to each well, and wash thoroughly for 3 times, 5 min each time.
- 4) Discard the supernatant, add 1mL of PBS (with 0.3% Triton X-100) to each well, and incubate 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) Discard the supernatant, add 1 mL of PBS (with 3% BSA) to each well, and wash thoroughly for 3 times, 5 min each time.

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

Ingredient	Sample size of 6-well plate						
	1	2	4	5	10	25	50
Click Reaction Buffer II	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
Alexa Fluor® 647 Azide	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.
- 3) Discard the supernatant, then add 500 µL of Click Reaction Solution to each well, shake gently to ensure that the Click Reaction Solution evenly covers the cells and incubate at RT for 30 min in the dark.
 - 4) Discard the supernatant, add 1 mL of PBS (with 3% BSA) to each well and wash for 3 times, 5 min each time.

4. Nuclear staining

- 1) Discard the supernatant, add 500 µL of DAPI working solution to each well, and incubate at RT for 5-10 min in the dark.
- 2) Discard the supernatant, add 1 mL of PBS (with 3% BSA) to each well and wash for 3 times, 5 min each time.

5. Analyze

Select an appropriate filter to observe the results under a fluorescence microscope.

Dye	Ex/Em (nm)	Filter Set
Alexa Fluor® 647	650/665	Cy5 Filter Set
DAPI	350/470	DAPI Filter Set

Note: 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 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 μ M EdU can be used as initial exploratory concentration.
4. Since the EdU labeling reaction is carried out in the cells and detected by fluorescence microscope, 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. Aliquot the Click Additive Solution and store at -20°C. If

5. 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.