

FN-Click EdU Cell Proliferation Flow Cytometry Assay Kit (Red, FineTest®647)

Catalog No.: FNCK108

Size: 50T/200T

Kit components:

Cat.	Reagents	50T	200T
FNCK108A	EdU(10mM)	200 μ L	800 μ L
FNCK108B	Click Reaction Buffer I	25mL	50mL*2
FNCK108C	FineTest®647 Azide I	50 μ L	200 μ L
FNCK108D	CuSO4	1mL*2	8mL
FNCK108E	Click Additive	220mg	220mg*4

Note: 50 T means that 50 samples can be tested with 6 well plates. EdU (10 mM) needs to be stored in aliquots for the first use(50 μ L/ vial is recommended or aliquot into smaller quantities according to experimental needs).

Storage:

Store at -20°C for 1 year. FineTest®647 Azide I needs to be stored away from light.

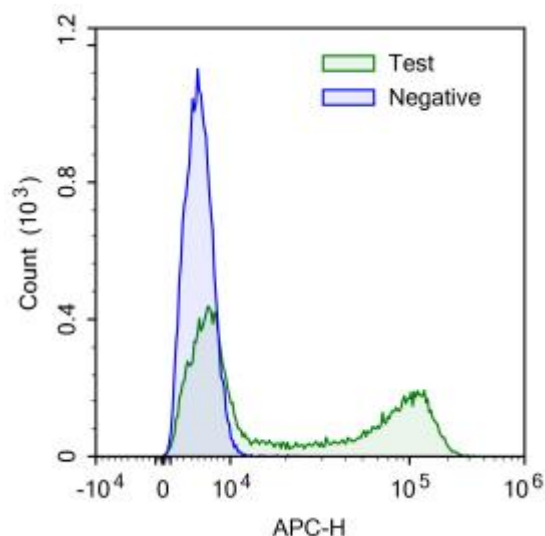
Description:

FN-Click EdU Cell Proliferation Flow Cytometry Assay kit (red, FineTest®647) is convenient and sensitive for proliferation detection of cell suspension samples. The results can be analyzed by flow cytometry.

Cell proliferation detection is widely used in the evaluation of cell activity, genotoxicity and efficacy of anti-tumor drugs. Direct detection of DNA synthesis in cells is considered to be the most accurate method to detect cell proliferation. The first widely used method to detect DNA synthesis in cells was the radionuclide incorporation method, but this method was greatly limited due to radioactive contamination and the difficulty of single-cell detection, and it was gradually replaced by the BrdU method based on antibody detection. 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, high detection sensitivity, is a new method to upgrade on the basis of BrdU method, will gradually replace BrdU method. EdU(5-ethynyl-2-deoxyuridine) is a thymine deoxyriboside analogue that can be incorporated into newly synthesized DNA in place of thymine deoxyriboside during DNA synthesis. On the other hand, the acetylene group on EdU can covalently react with a fluorescently labeled small molecule azide probe to form a stable triazole ring catalyzed by a monovalent copper ion, which is a very rapid reaction known as the Click reaction. Through the click reaction, the newly synthesized DNA is labeled with a corresponding fluorescent probe, so that the proliferating cells can be detected using appropriate fluorescence detection equipment.

Test results refer to the figure below:



Test: HeLa cells were treated with 10 μ M EdU for 3 hours

Negative: HeLa cells without EdU

Materials Not Supplied:

1. Reagents: PBS (with 1% BSA) (pH 7.2~7.6); Permeabilization buffer: 1% Saponin (dissolved in PBS, pH 7.2~7.6); 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 -20° C. (It is recommended to open a new vial of Click Additive after using one tube).

Assay Protocol:

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

5)Centrifuge at $300\times g$ for 5 min, discard the supernatant, then resuspend the cells with 1mL PBS (with 1% BSA) and mix fully.

6)Centrifuge at $300\times g$ 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 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
FineTest®647 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

Note: 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; 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: The maximum excitation wavelength of FineTest® 647 is 650 nm and the maximum emission wavelength is 670 nm; 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

Note:

1. 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.
2. 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.
3. Aliquot the Click Additive Solution and store at -20°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.
4. 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.
5. For your safety and health, please wear a lab coat and disposable gloves.
6. This kit is for scientific research only.