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

Catalog No.: FNCK112

Size: 50T/200T

Kit components:

Cat.	Reagents	50T	200T	Storage
FNCK112A	EdU(10mM)	200 μL	800 μL	-20℃
FNCK112B	Click Reaction Buffer II	25mL	50mL*2	-20℃
FNCK112C	FineTest®647 Azide II	50 μL	200 μL	-20℃, shading light
FNCK112D	CuSO4	1mL*2	8mL	-20℃
FNCK112E	Click Additive	220mg	220mg*4	-20℃
FNCK112F	DAPI Reagent	1mL	1mL*4	-20℃, shading light

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 μL/ vial is recommended or aliquot into smaller quantities according to experimental needs).

Storage:

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

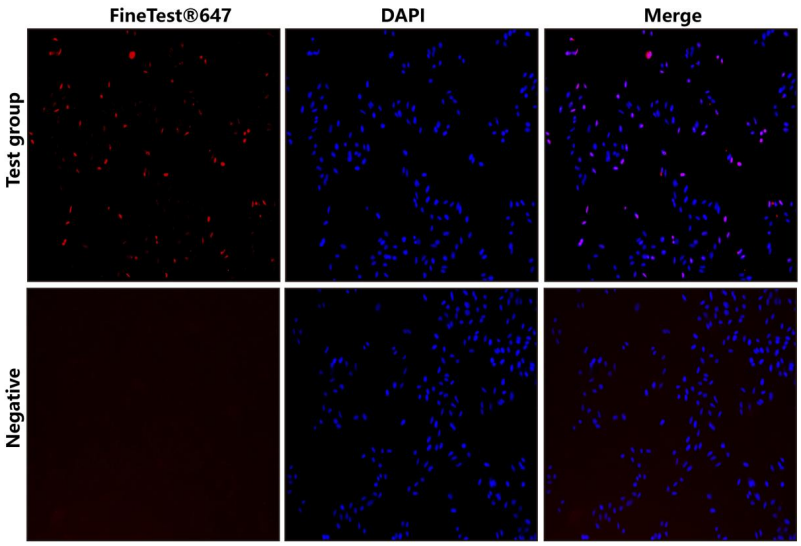
Description:

FN-Click EdU Cell Proliferation Imaging Assay Kit (red, FineTest®647) is convenient and sensitive for proliferation detection of cell slides and smears. The results can be analyzed by fluorescence microscope.

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 μM EDU for 3 hours

Negative:Hela cells without EDU

Materials Not Supplied:

1. Reagents: PBS（pH7.2~7.6); PBS (with 3% BSA) (pH7.2~7.6); Permeabilization buffer: 0.3% TritonX-100 (dissolved in PBS, pH7.2~7.6); Fixation buffer: 4% Polyformaldehyde (dissolved in PBS, pH7.2~7.6); 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 to 96 μL PBS and mix well. Prepare the fresh solution before use.

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 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)After incubation, discard the supernatant.

2) Add 1mL of 4% Polyformaldehyde (dissolved in PBS) to each well, incubate at RT for 15 min, and then remove the 4% Polyformaldehyde.

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 X100) 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 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						
	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
CuSO4	40 μL	80 μL	160 μL	200 μL	400 μL	1 mL	2 mL
FineTest®647 Azide II	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

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.

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
FineTest®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 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

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 μM EdU can be used as initial exploratory concentration.

2. 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.

3. 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.

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.