Product Information

YF®488/555/594/647A Click-iT EdU Flow Cytometry Assay Kits

Catalog Number: C6019 (YF®488), C6020 (YF®555), C6021 (YF®594), C6022 (YF®647A)

Product Size: 20 T, 50 T

Contents:

Component	20 T	50 T	Storage	Stability
A. 10 mM EdU	0.4 mL	1 mL	-20℃	When stored as directed, product is stable for at least 12 months.
B. YF®488/555/594/647A Azide	100 µL	250 µL	-20℃, protect from light	
C. $CuSO_4$	0.8 mL	2 mL	2-8℃	
D. Click-iT EdU Buffer Additive	60 mg	150 mg	2-8℃	

Specification: the above reaction times(T) are for cells cultured on 6 well plate. For specific reagent dosage of other containers, please refer to Schedule 1 (Dosage reference of EdU and dye reaction solution).

Fluorescence spectrum parameters: YF®488 Azide: 495/519 nm; YF®555 Azide: 555/565 nm; YF®594 Azide: 594/617 nm; YF®647A Azide: 650/670 nm

Reagents required:

10 mM PBS, pH 7.2-7.6

Paraformaldehyde (4% paraformaldehyde in PBS)

0.5% Triton X-100 in PBS

1% BSA in PBS, pH7.4

Deionized water

Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 12 months. After unsealing, the storage temperature is shown in the table above.

Description

Detection of cell proliferation is a basic experimental method to evaluate cell health, genotoxicity and antitumor effect. Previously, the most accurate method to detect cell proliferation was BrdU method, and EdU method was a revolutionary breakthrough of BrdU method. EdU (5-ethynyl-2-deoxyuridine) is a pyrimidine analogue that can be integrated into the DNA double strand during DNA synthesis. EdU method is based on the "Click" reaction, an atomic covalent reaction of azides and alkynes catalyzed by copper.

In this kit, EdU is a compound contains alkyne, YF®488/555/594/647A Azide dye reagent contains azide compound. EdU method is a rapid and effective method for cell proliferation and easy to use. Only a small amount of azide dye is needed to mark the integrated EdU. After fixation with paraformaldehyde and penetration with Triton X-100, detection reagent can enter cell without DNA denaturation. However, the BrdU method needs DNA denaturation (such as acid

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denaturation, heat denaturation or DNase digestion) to expose BrdU, so as to facilitate the binding of BrdU antibody.

This kit contains all components needed for EdU assay, which can analyse cell proliferation and cell cycle.

Protocol

1. Labeled cells with EdU

Note: The labeling concentration of EdU should be optimized according to different cell type, we recommend to explore with initial concentration of 10 μM. Cell culture medium, cell growth density, cell type and other experimental conditions may affect the labeling effect of cells. In the pre experiment, we suggest to set a series of EdU concentration gradients to determine the optimal cell experimental concentration. Please refer to Schedule 2 to get incubate concentration and time of EdU.

1.1 Inoculate 1 × 10^5-3 × 10^6 cells into 6 well plate for drug treatment or other stimulation treatment.

1.2 It is recommended to conduct pre experiment with initial working concentration of 10 μM. Cell culture medium, cell growth density, cell type and other experimental conditions may affect the labeling effect of cells. In pre experiment, we suggest to set a series of EdU concentration gradients to find out the most suitable experimental concentration for your cells. The negative control group was not treated with EdU.

1.3 Incubate cells with suitable conditions and time, time of incubation depend on cell growth rate. The pulsed labeled cells incubated by EdU can be used to study cell cycle dynamics.

Note: the concentration of EdU is related to incubation time. High concentration should be used for short-term incubation (< 2h), such as 10-50 μM, and low concentration for long-term incubation (> 24h), such as 1-10 μM).

2. Cell fixation and permeabilization

2.1 Collecting cells after incubation, washed with 1% BSA in PBS, then centrifuged and remove the medium.

2.2 Add 1 mL 4% neutral paraformaldehyde to resuspend cells, incubate at room temperature for 15 min, and then remove the fixing solution.

2.3 Wash cells twice with 1 mL 1% BSA in PBS .

2.4 Remove the washing solution, add 1 mL 0.5% Triton X-100 in PBS, and incubate at room temperature for 20 min.

3. Detection of EdU

Note: this protocol is provided for 1 mL Click-iT reaction mixture for each well reaction. You can adjust the proportion to reduce the volume of solution according to your own sample situation.

3.1 Prepare 5 × Click-iT EdU Reaction Additive storage solution (100 mg/mL): add 0.6 mL deionized water to Click-iT EdU Buffer Additive tube(component D, 60 mg), mix thoroughly to make sure that all powder completely dissolved (Note: for other specifications, due to the different amount of component D, volume of added deionized water needs to be expanded in proportion). After use, the remaining storage solution can be stored for one year at ≤ -20 ℃ . Once the solution turns to brown, it means that the effective components can not be degraded again (Note: the components D of different specifications are dissolved in deionized water according to this proportion, and prepared into 5 × storage solution for standby).

3.2 Prepare 1× Click-iT EdU Buffer Additive: dilute 5 × storage solution to 1 × with deionized water. The solution should be fresh and used up on the same day.

3.3 Prepare Click-iT reaction mixture according to Table 1. The components required to be added in Table 1 are very important for the reaction, otherwise the reaction cannot be carried out effectively.

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Table 1. Click-iT Reaction Mixture

Reaction component	For each reaction
PBS, D-PBS or TBS	875 μL
$CuSO_4$	20 μL
YF®488/555/594/647A Azide	5 μL
1× Click-iT EdU Buffer Additive	100 μL
Total volume	1 mL

3.4 Remove the 0.5% Triton X-100 in PBS, wash twice with 1 mL 1% BSA in PBS for each well, and remove the washing solution.

3.5 Add 1 mL Click-iT reaction mixture to each well and shake the plate briefly to ensure that the reaction mixture covers cells evenly.

3.6 Incubate at room temperature in dark for 30 min.

3.7 Remove the reaction mixture, wash with 1mL 1% BSA in PBS for each tube, then remove the washing solution. 1% BSA in PBS was used to suspend the cells again, then analysis by flow cytometry. (Note: please refer to 4.1-4.3 if other signs need to be tested at the same time.).

4. Intracellular antigen labeling (optional step)

4.1 Add incubation working solution of required antibody and mix well.

4.2 Cells were incubated with antibody at suitable temperature and time in dark.

4.3 Wash each tube with 0.5% Triton X-100, collect cells and remove supernatant.

4.4 1% BSA in PBS was used to suspend the cells again, then analysis by flow cytometry.

5. DNA content calculation (optional steps)

5.1 If necessary, add appropriate RNase to each tube and mix evenly.

5.2 Add appropriate DNA staining solution to each tube, mix evenly, and incubate in dark for 10-15 min.

5.3 Analysis by flow cytometry.

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Appendix:

Schedule 1. Reference usage of medium with EdU and dye reaction solution

	96 well plate	**48** well plate	**24** well plate	**12** well plate	**6** well plate	**5.5** cm Petri dish
medium with EdU	100 μL	150 μL	200 μL	500 μL	1 mL	2 mL
dye reaction solution	100 μL	150 μL	200 μL	500 μL	1 mL	2 mL

Schedule 2. Reference concentration and time of EdU incubation

PubMed ID	Reference	Cell Line	Concentration	Time
18272492	Salic A,*et al.*PNAS.2008	NIH3T3,Hela	10 nM~10 μM	1 hr
18521918	Cappella P, *et al.* Cytometry A.2008	HL-60,A2780, U2OS	1~10 μM	30 min
18996411	Chehrehasa F, *et al.* Neurosci Methods.2009	Neurospheres	1~20 μM	24 hr
19179371	Limsirichaikul S, *et al.* Nucleic Acids Res.2009	Primary fibroblasts	10 μM	1,2,4 hr
19253396	Warren M, *et al.* Dev Dyn.2009	Chick embryos	10 μM~2 mM	4 hr
19647746	Yu Y, *et al.* J Immunol Methods.2009	Spleen cells	50 μM	24 hr
19544417	Momcilovic O, *et al.* Stem Cells.2009	Human ES cells	10 μM	30 min
20080700	Cinquin O, *et al.* PNAS.2010	emb-30	1 μM	12 hr
20025889	Han W, *et al.* Life Sci.2009	VSMC	50 μM	2 hr
20659708	Huang C, *et al.* J Genet Genomics.2010	ESC	50 μM	2 hr
21310713	Hua H, *et al.* Nucleic Acids Res.2011	Fission yeast strains	10 μM	3 hr
20824490	Lv L, *et al.* Mol Cell Biochem.2011	EJ cells	50 μM	4 hr
21248284	Yang S, *et al.* Biol Reprod.2011	GC cells	50 μM	2 hr
21227924	Zhang YW, *et al.* Nucleic Acids Res.2011	U2OS, HT29	30 μM	90 min
21829621	Guo T, *et al.* PloS One. 2011	HIT-T15	50 μM	4 hr
21980430	Zeng T, *et al.* PloS One. 2011	MCF-10A	25 μM	2 hr
22012572	Ding D, *et al.* Int Orthop.2011	C3H10T1/2	10 μM	24 hr
22000787	Zeng W, *et al.* Biomaterials.2011	EPC	50 μM	4 hr
21913215	Xue Z, *et al.* J Cell Biochem.2011	SGC7901	25 μM	24 hr
22016038	Peng F, *et al.* Lasera Med Sci.2011	MSC	50 μM	2 hr
21878637	Li D, *et al.* J Biol Chem.2011	HCC	50 μM	2 hr

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