Product Information

YF®488/555/594/647A Click-iT EdU Imaging Kits

Catalog Number and Product Size:

Catalog Number	Product name	Size
C6015S	YF® 488 Click-iT EdU Imaging Kits (Green fluorescence)	20T
C6015M		100T
C6015L		500T
C6015XL		1000T
C6016S	YF® 555 Click-iT EdU Imaging Kits (Orange red fluorescence)	20T
C6016M		100T
C6016L		500T
C6016XL		1000T
C6017S	YF® 594 Click-iT EdU Imaging Kits (Red fluorescence)	20T
C6017M		100T
C6017L		500T
C6017XL		1000T
C6018S	YF® 647A Click-iT EdU Imaging Kits (Far-Infrared fluorescence)	20T
C6018M		100T
C6018L		500T
C6018XL		1000T

Contents:

Size / Component	20T	100T	500T	1000T	Storage	Stability
A. 10 mM EdU	40 µL	0.2 mL	1 mL	2×1 mL	-20°C	When stored as directed, product is stable for at least 12 months.
B. YF® 488/555/594/647A Azide	4 µL	20 µL	100 µL	200 µL	-20°C, protect from light	
C. 10× Click-iT EdU Reaction Buffer	200 µL	1 mL	5 mL	10 mL	2-8°C	
D. $CuSO_4$	100 µL	0.5 mL	2.5 mL	5 mL	2-8°C	
E. Click-iT EdU Buffer Additive	6 mg	30 mg	150 mg	2×150 mg	2-8°C	
F. Hoechst 33342	5 µL	25 µL	125 µL	250 µL	2-8°C	

Specification: the above reaction times(T) are for cells cultured on 96 well plate. For specific reagent dosage of other containers, please refer to Schedule 1 (Dosage reference of EdU and dye reaction solution).

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Fluorescence spectrum parameters: YF®488 Azide: 495/519 nm; YF®555 Azide: 555/565 nm; YF®594 Azide: 590/617 nm; YF®647A Azide: 650/670 nm; Hoechst 33342: 350/461 nm, bound to DNA.

Reagents required:

10 mM PBS, pH7.2-7.6

Paraformaldehyde (4% paraformaldehyde in PBS)

2 mg/mL glycine solution (prepared with deionized water)

0.5% Triton X-100 in PBS

3% BSA in PBS, pH7.2-7.6

Coverslip (18×18 mm)

Storage

Store at -20℃ and protect from light. Expiration date marked on the outer packing. 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 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. For staining the nucleus, the kit provided nuclear dye Hoechst 33342.

Protocol

Note: Refer to Product Information of EdU(Catalog Number: E6032) to get animal imaging experiment protocol. The following protocol is showing for cell imaging.

1. Labeled cells with EdU

Note: The labeling concentration of EdU should be optimized according to different cell type, we recommend to explore with the 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 find out the optimal cell experimental concentration. Please refer to Schedule 2 to get incubate concentration and time of EdU.

(1) Inoculate 4×10^3-1×10^5 cells into 96 well plate for drug treatment or other stimulation treatment.

(2) Prepare 2 × EdU working solution (component A): dilute the 10 mM stock solution to appropriate working concentration with complete medium. It is recommended to conduct pre experiment with initial working concentration of 10 μM.

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(3) Preheat 2 × EdU working solution, and add it into cell culture medium in equal volume to obtain final concentration to 1 × (for example, add 100 μL 20 μM 2 × EdU working solution into 100 μL cell culture medium to obtain 10 μM final concentration).

(4) 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.

2. Cell fixation and permeabilization

(1) After incubation, remove the medium. Add 50 μL 4% neutral paraformaldehyde to each well, incubate at room temperature for 15-30 min, and then remove the fixing solution.

(2) Add 50 μL 2 mg/mL glycine solution to each well, incubate at room temperature for 5 min to neutralize the remaining stationary solution.

(3) Wash cells twice with 0.1 mL 3% BSA in PBS per well.

(4) Remove the washing solution, add 0.1 mL 0.5% Triton X-100 in PBS to each well, and incubate at room temperature for 20 min.

3. Detection of EdU

Note: this protocol is provided for 100 μL 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.

(1) Prepare 1× Click-iT EdU Reaction Buffer: dilute 10× Click-iT EdU Reaction Buffer(component C) with deionized water for 10 times.

(2) Prepare 5 × Click-iT EdU Reaction Additive storage solution (100 mg/mL): add 0.3 mL deionized water to Click-iT EdU Buffer Additive tube(component E, 30 mg), mix thoroughly to make sure that all powder completely dissolved (Note: for other specifications, due to the different amount of component E, 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 E of different specifications are dissolved in deionized water according to this proportion, and prepared into 5 × storage solution for standby).

(3) 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.

(4) 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.

Table 1. Click-iT Reaction Mixture

Reaction component	For 10 wells
1× Click-iT EdU Reaction Buffer	860 μL
$CuSO_4$	40 μL
YF®488/555/594/647A Azide	2 μL
1× Click-iT EdU Buffer Additive	100 μL
Total volume	1 mL

(5) Remove the 0.5% Triton X-100 in PBS, wash twice with 0.1 mL 3% BSA in PBS for each well, then remove the washing solution.

(6) Add 0.1 mL Click-iT reaction mixture to each well and shake the plate briefly to ensure that the reaction mixture covers cells evenly.

(7) Incubate at room temperature in dark for 30 min.

(8) Remove the reaction mixture, wash twice with 0.1 ml 3% BSA in PBS for each well, then remove the washing solution.

For nuclear staining, DNA can be restained with Hoechst 33342.

4. Nuclear staining

(1) Wash each well with 0.1 mL PBS and remove the washing solution.

(2) Diluted Hoechst 33342 (component F) with PBS for 2000 times to get 1 ×Hoechst 33342 solution. The final concentration is 5 μg/mL.

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(3) Add 0.1mL 1 × Hoechst 33342 solution to each well, and incubate at room temperature in dark for 15-30 min. Remove Hoechst 33342 solution.

(4) Wash each well twice with 0.1 mL PBS, remove washing solution.

5. Imaging and analysis

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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