Cell Proliferation EdU Image Kit (Green Fluorescence)

Cat #: KTA2030 Size:100 T/500 T

	Cell Proliferation EdU Image Kit (Green Fluorescence)		
REF	**Cat #:** KTA2030	LOT	**Lot #:** Refer to product label
	Applicable samples: Adherent cells, suspension cells, Living animals		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The detection of cell proliferation is of utmost importance for assessing cell health, determining genotoxicity or evaluating anticancer drugs. Until now, measuring DNA synthesis directly is most accurate method of doing it, normally performed by incorporation of the nucleoside analog like [3H] thymidine or 5-bromo-2'-deoxyuridine to cells during replication, and then detected or visualized by autoradiography or with an anti-BrdU-antibody respectively. Cell Proliferation EdU Image Kit (Green Fluorescence) is a novel alternative to the BrdU assay. EdU (5-ethynyl-2´-deoxyuridine) is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis. Comparing to BrdU assays, the EdU-Click Assays are not antibody based and therefore do not require DNA denaturation (typically using HCl or heat or digestion with DNase) for detection of the incorporated nucleoside. Detection is based on a click reaction, a copper-catalyzed covalent reaction between an azide and an alkyne, is complete within 30 min.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	100 T	500 T	
EdU (10 mM)	100 μL	500 μL	-20℃
BSA Wash Solution (5×)	12 mL	60 mL	-20℃
AbFluor 488 azide	20 μL	100 μL	-20℃, protected from light
Reaction Buffer (10×)	1 mL	5 mL	4℃
Copper Reagent	0.4 mL	2 mL	4℃
Reducing Agent	100 mg	5×100 mg	-20℃
Hoechst 33342 (1,000×)	12 μL	60 μL	-20℃, protected from light
Hydroxyurea	10 μL	50 μL	4℃, protected from light

Materials Required but Not Supplied

- Cryogenic centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water
- Fixation solution (3.7% formaldehyde in PBS)
- Permeable agent (for example, 0.5% Triton X-100 in PBS)

Reagent Preparation

EdU (10 mM): Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

BSA Wash Solution (1×): Add PBS to dilute BSA Wash Solution (5×) into 1×work solution (final concentration is 3% BSA/ PBS), and mix well. The solution can be stored stably for 6 months at -20℃ after aliquoting.

AbFluor 488 Azide: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

10×Reaction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Copper Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Preparation of Reducing Agent (10×): Add deionized water to Reducing Agent to prepare Reducing Agent (10×) with a final concentration of 100 mg/mL and mix until the compound is completely dissolved. The solution can be stored stably for 6 months at -20℃ after aliquoting. If the solution turns brown, the components have been degraded and it is not recommended to continue using it.

Hoechst 33342 (1×)： Use PBS to dilute 1×Hoechst 33342 working solution at a ratio of 1:1,000 before use. Stored at -20℃, protected from light after aliquoting.

Hydroxyurea： DNA synthesis inhibitor. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light after aliquoting.

Assay Procedure

A. Labeling of cells with EdU

In this experiment, adherent cells cultured in 96-well plate were taken as an example. Suspension cells can be smeared after incubating EdU (adding appropriate amount of cells to glass slides and baking with alcohol lamp until dry). After smear, the staining procedure is the same as that of adherent cells.

1. Place suitable coverslips in the plate, seed an appropriate number of cells, and allow them to grow to the desired density after processing the cells.
2. Optional step: setting of negative control: add DNA synthesis inhibitor Hydroxyurea before EdU incubation, add directly into the negative control hole according to the proportion of 1:1000, mix and incubate for 0.5 h.
3. 2×EdU working solution (20 μM) was prepared with 10mM EdU solution in medium. The recommended final concentration of EdU is 10 μM, and 2×EdU working solution (20 μM) can be obtained by diluting 10mM EdU with cell culture medium 1:500.
4. The same volume of 2×EdU solution preheated (37℃) was added to the medium containing test cells to change the final concentration of EdU in the 96-well plate to 1×.

Note: it is recommended not to change all media because it will affect the rate of cell proliferation; it is recommended that the initial concentration of EdU is 10 μM.

5. Incubate cells for 2 h under the most suitable conditions (according to the time of cell expansion, the incubation time of general tumor cells is 2 h).
6. After incubation, the culture medium was removed, and 0.1mL fixing solution (PBS containing 3.7% formaldehyde) was added to each well. 15min was incubated at room temperature.
7. Remove the fixative and wash the cells in each well with 0.1 mL of BSA Work Solution (1×) for 5 min. Repeat 3 times.
8. Remove the detergent, add 0.1 mL permeable agent (PBS containing 0.5%Triton X-100) to each well, and incubate at room temperature for 15 min.
9. Remove the permeable agent and wash the cells in each well with 0.1 mL of BSA Work Solution (1×) for 5 min. Repeat 2 times.

10. Turn to step C.

B. EdU labeling living animals

This experiment takes 6-week-old mice as an example, the labeling of EdU in other animals, please refer to the relevant literature.

1. For mice, according to the dosage of 10-200 mg/kg, EdU can be prepared with PBS into a certain concentration, intraperitoneal injection, local injection of specific tissues or organs, or added to drinking water. The specific dosage is related to the type, weight and mode of use of the animals used, we can refer to the relevant literature, so it is recommended to explore the concentration of EdU for the first use, or directly use the concentration of 50 mg/kg for testing. If you have previously used BrdU for experiments, you can refer to the final concentration of BrdU as the final concentration of EdU. EdU needs to be purchased separately.

2. Optional step: setting of negative control: DNA synthesis inhibitor Hydroxyurea was added during EdU treatment and prepared with EdU solution at the concentration of 1000 mg/kg. Hydroxyurea needs to be purchased separately.

3. After 24 h or after the appropriate time determined according to the specific experiment, the mice were killed quickly, the necessary tissue was removed, and the frozen sections or paraffin sections were made according to the routine steps. The time marked by EdU can also be adjusted by referring to the relevant literature.

4. For frozen sections:

(1) Add appropriate amount of fixed solution (PBS containing 3.7% formaldehyde) and incubate 15min at room temperature.

(2) Remove the fixative and wash the cells in each well with 0.1 mL of BSA Work Solution (1×) for 5 min. Repeat 3 times.

(3) Remove the detergent, add 0.1 mL permeable agent (PBS containing 0.5% Triton X-100) to each well, and incubate at room temperature for 15 min.

(4) Remove the permeable agent and wash the cells in each well with 0.1 mL of BSA Work Solution (1×) for 5 min. Repeat 2 times.

(5) Antigen repair (optional): If immunofluorescence staining of the target protein is needed at the same time, and antigen repair is necessary, appropriate antigen repair solution or self-made appropriate antigen repair solution can be used for antigen repair.

(6) Turn to step C.

5. For paraffin sections:

(1) Dewaxing: dewaxing in xylene for 5-10 min, then changing to fresh xylene, then dewaxing for 5-10 min. 5 min of anhydrous ethanol and 3 min of anhydrous ethanol. 95% ethanol 3 min. 85% ethanol 3 min. 75% ethanol 3 min. 50% ethanol 3 min. PBS 5 min.

(2) Antigen repair (optional): If immunohistochemical staining of the target protein is needed at the same time, and antigen repair is necessary, appropriate antigen repair solution or self-made appropriate antigen repair solution can be used for antigen repair.

Note: If protease K or trypsin is used for antigen repair, it must be washed repeatedly, otherwise the residual enzyme will seriously interfere with the follow-up labeling reaction.

(3) Turn to step C.

C. EdU detection

Note: In this step, 100 μL reaction mixture is used per well. For other orifice plates or slices, the volume of the reaction mixture can be adjusted according to the actual situation, but the reaction components must be added in proportion.

1. Prepare the Click-iT reaction mixture according to the table below.

Note: It is important to add the ingredients in the order listed in the table; otherwise, the reaction will not proceed optimally. Use the Click-iT reaction mix within 15 min of preparation.

Component	Volume
Deionized Water	758 μL
Reaction Buffer (10×)	100 μL
Copper Reagent	40 μL
AbFluor 488 Azide	2 μL
Reducing Agent (10×)	100 μL

Total Volume	1 mL

2. Remove the solution, add 100 μL of Click-iT reaction mix to each sample and incubate the cells for 30 min at room temperature and protected from light.

3. Remove the reaction mixture, wash the cells in the well with 0.1 mL BSA Wash Solution (1 ×) for 5 min, and remove the washing solution.

4. Optional steps: nuclear staining (1×Hoechst 33342) or antibody labeling.

Important tip: Avoid light in the process of incubation. If you do not need other staining, please image and analyze directly after hatching.

5. The labeled DNA in the sample was analyzed by fluorescence microscope (Ex/Em=501/525nm) and the nucleus was detected by Ex/Em=360/460nm.

Precautions

1. To avoid cross-contamination, tip changes when adding different samples and different reagents.
2. Make sure all components and equipment are at the right temperature before the experiment starts.
3. Fluorescent dyes have quenching problems, please pay attention to avoid light as much as possible to slow down fluorescence quenching.
4. For your safety and health, please wear a lab coat and wear disposable gloves.

Highlight moment: In addition to DNA structural changes to detect cell proliferation, in most cases, cell proliferation is evaluated by detecting the content of NADH in the cytoplasm. The structural change of DNA is the early event of cell proliferation (KTA2030), and the structural change of DNA leads to the change of NADH content (BMU106-CN). In addition, to comprehensively detect the state of cells, it is also necessary to analyze from the perspective of apoptosis (KTA2010, KTA0002) and cell senescence (KTA3030). Scan the QR code on the right and follow the official account of Abbkine to learn more about Abbkine products.

Recommended Products

Catalog No.	Product Name
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA2011	One-step TUNEL Apoptosis Assay Kit (Orange Fluorescence)
KTA2020	Cell Cycle Staining Kit
KTA2031	EdU Cell Proliferation Image Kit (Orange Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.