Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

EdU Cell Proliferation Image/Flow Cytometry Assay Kit (Orange Fluorescence)

Cat #: KTA2031 Size:200 T/1000 T

	Cell Proliferation EdU Image Kit (Orange Fluorescence)		
REF	**Cat #:** KTA2031	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

Detection of cell proliferation is essential for assessing cell health, determining genotoxicity, or evaluating anticancer drugs. Currently, direct measurement of DNA synthesis is the most accurate method. Nucleoside analogs (e.g., [^{3}H]thymidine or 5-bromo-2′-deoxyuridine, BrdU) are incorporated into cells during replication and subsequently detected by autoradiography or using anti-BrdU antibodies.

The EdU Cell Proliferation Imaging Analysis Kit (Orange Fluorescence) is a novel alternative to the BrdU assay. EdU (5-ethynyl-2′-deoxyuridine) is a nucleoside analog of thymidine that can be incorporated into newly synthesized DNA in place of thymidine during DNA synthesis. Compared with the BrdU method, the EdU-Click detection is not antibody-based, thus eliminating the need for DNA denaturation (typically performed using hydrochloric acid, heat, or DNase digestion) to detect the incorporated nucleoside. The detection is based on the Click reaction, a copper-catalyzed covalent reaction between an azide and an alkyne, and the entire reaction can be completed within 30 min.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	200 T	1000 T	
EdU (10 mM)	100 μL	500 μL	-20℃
Permeabilization Reagent	240 mg	1200 mg	-20℃, protected from light, For flow cytometry detection
BSA Wash Solution (5×)	12 mL	60 mL	-20℃
AbFluor 545 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	20 μL	100 μL	4℃, protected from light

Materials Required but Not Supplied

·Cryogenic centrifuge, Fluorescence Microscopy and Flow Cytometry, EdU detection by fluorescence microscopy: Ex/Em = 546/565 nm,Hoechst 33342: Ex/Em = 360/460 nm,EdU detection by flow cytometry: PE channel

·Precision pipettes, disposable pipette tips

·Deionized water

·Fixation solution (3.7%-4% formaldehyde or paraformaldehyde in PBS)

·Permeable agent (for example, 0.3% or 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.

Permeabilization Buffer: For flow cytometry detection. Prepare immediately before use. For the 200 T kit, add 24 mL of PBS to the Permeabilization Reagent; for the 1000 T kit, add 120 mL of PBS to the Permeabilization Reagent to prepare Permeabilization Buffer at a final concentration of 10 mg/mL. Mix until the compound is completely dissolved before use. Aliquot after use and store at -20°C, protected from light.

AbFluor 545 Azide: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Reaction Buffer (10×): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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

Preparation of Reducing Agent (10×): Add 1 mL 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×)： Dilute Hoechst 33342 (1,000×) with PBS 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. For adherent cells (analyzed by fluorescence microscopy)

1. Taking adherent cells cultured in a 96-well plate as an example, seed an appropriate number of cells into the plate, allow them to grow to the desired density, and then treat the cells.
2. Optional step: Setting up a negative control: Before EdU incubation, add the DNA synthesis inhibitor hydroxyurea directly to the negative control wells at a ratio of 1:500, mix well, and incubate for 0.5–1 h.
3. Prepare 2×EdU working solution (20 μM) by diluting the 10 mM EdU solution with cell culture medium. Dilute the 10 mM EdU 1:500 with cell culture medium to obtain the 2×EdU working solution (20 μM).
4. Add an equal volume of pre-warmed (37°C) 2×EdU solution to the culture medium containing the experimental cells, so that the final concentration of EdU in the 96-well plate becomes 1×.

Note: It is not recommended to completely replace the culture medium, as this may affect cell proliferation efficiency. The recommended starting working concentration of EdU is 10 μM.

5. Incubate the cells for 2 h under optimal conditions (determined based on cell doubling time; generally, the incubation time for tumor cells is 2 h).
6. After incubation, remove the medium and add 100 μL of fixative (PBS containing 3.7%–4% formaldehyde or paraformaldehyde) to each well. Incubate at room temperature for 15 min.
7. Remove the fixative and wash the cells in the wells with 100 μL PBS for 5 min. Repeat 3 times.
8. Remove the wash solution and add 100 μL of permeabilization solution (PBS containing 0.3% Triton X-100) to each well.

Incubate at room temperature for 30 min.

9. Remove the permeabilization solution and wash the cells in the wells with 100 μL BSA Wash Solution (1×) for 5 min. Repeat 3 times.

10. Turn to step D.

Note: For cells grown on coverslips and other plate formats, the volumes of fixative and permeabilization solution may be adjusted according to actual conditions.

B. For non-adherent cells (analyzed by flow cytometry)

1. Culture cells to the optimal density (approximately 5 × 10^6 cells/mL).

2. Optional step: Setting up a negative control: Before EdU incubation, add the DNA synthesis inhibitor Hydroxyurea directly to the negative control wells at a ratio of 1:500, mix well, and incubate for 0.5–1 h.

3. Prepare a 2×EdU working solution (20 μM) by diluting the 10 mM EdU stock solution with cell culture medium. Dilute the 10 mM EdU 1:500 in cell culture medium to obtain the 2×EdU working solution (20 μM).

4. Add an equal volume of pre-warmed (37°C) 2×EdU working solution to the medium containing the test cells, resulting in a final EdU concentration of 1×.

Note: It is not recommended to completely replace the culture medium, as this may affect cell proliferation efficiency. The recommended starting working concentration of EdU is 10 μM.

5. Incubate the cells under optimal conditions for 2 h (the incubation time can be adjusted according to the cell doubling time; for most tumor cells, an incubation time of 2 h is typical).

6. Centrifuge at 300 g for 5 min, collect the cells, and wash twice with PBS.

7. Add 500 μL of fixative solution (PBS containing 3.7%–4% formaldehyde or paraformaldehyde) and fix at room temperature for 20 min.

8. Centrifuge at 800 g for 5 min, remove the fixative, collect the cells, resuspend in 1 mL of PBS, and repeat the wash twice.

9. Centrifuge at 800 g for 5 min, remove the wash solution, collect the cells, resuspend in 500 μL of Permeabilization Buffer, and permeabilize at room temperature for 20 min.

10. Turn to step D..

C. 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, 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 1,000 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%-4% formaldehyde or paraformaldehyde) and incubate 15 min at room temperature.

(2) Remove the fixative and wash the cells in each well with an appropriate amount 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 an appropriate amount 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 D.

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 D.

D. EdU detection

1. Fluorescence Microscopy Analysis

Note:

1). In this step, using a 96-well plate as an example, add 50 μL of Click-iT working solution per well. For 48-, 24-, 12-, and 6-well plates, the corresponding volumes of Click-iT working solution are 70 μL, 100 μL, 200 μL, and 500 μL, respectively. If a coverslip is placed in the well during cell seeding, double the Click-iT working solution volume. For example, for 24-, 12-, and 6-well plates, the working solution volume can be increased to 200 μL, 400 μL, and 1 mL, respectively, to ensure complete coverage of the sample. For other plate formats or tissue sections, adjust the working solution volume proportionally according to your experimental needs.

2). When seeding cells,If a coverslip is not used, cells attach directly to the bottom of the culture plate; observation with an inverted fluorescence microscope is recommended. If a coverslip is used, after staining is complete, carefully remove the coverslip, mount it cell-side down on a clean glass slide using aqueous mounting medium or antifade mounting medium, and then observe with an fluorescence microscope.

(1). Prepare the Click-iT working solution according to the table below.

Note: Add the components in the order listed in the table, otherwise the reaction will not yield optimal results. The prepared Click-iT working solution must be used within 15 min.

Component	Samples per 96-well plate						
	1	2	4	5	10	25	50
Deionized Water	37.9 μL	75.8 μL	151.6 μL	189.5 μL	379 μL	947.5 μL	1,895 μL
Reaction Buffer (10×)	5 μL	10 μL	20 μL	25 μL	50 μL	125 μL	250 μL
Copper Reagent	2 μL	4 μL	8 μL	10 μL	20 μL	50 μL	100 μL
AbFluor 545 Azide	0.1 μL	0.2 μL	0.4 μL	0.5 μL	1 μL	2.5 μL	5 μL
Reducing Agent (10×)	5 μL	10 μL	20 μL	25 μL	50 μL	125 μL	250 μL
Total Volume	50 μL	100 μL	200 μL	250 μL	500 μL	1,250 μL	2,500 μL

(2). Add 50 μL of Click-iT reaction cocktail to each sample and incubate for 30 min at room temperature, protected from light.

(3). Remove the Click-iT reaction cocktail, wash the cells in the well with 100 μL of BSA Wash Solution (1×) for 5 min, and then remove the wash solution.

(4). Optional step: Perform nuclear staining (1× Hoechst 33342, incubate for 10 min at room temperature, protected from light) or antibody labeling.

Important tip: Samples must be protected from light during all incubation steps. If no additional staining is required, proceed directly to imaging and analysis after incubation.

(5). For cell coverslips, place a drop of aqueous mounting medium or antifade mounting medium on a glass slide, and mount the coverslip with the cells facing down. For paraffin-embedded sections, frozen sections, and other tissue samples, apply a drop of aqueous mounting medium or antifade mounting medium onto the tissue section and seal with a coverslip. Observe under a fluorescence microscope. For cells cultured in multiwell plates or culture dishes, add an appropriate volume of PBS to immerse the cells, then image using a fluorescence microscope. Analyze the labeled DNA in the samples using a fluorescence microscope with Ex/Em = 546/565 nm, and detect cell nuclei using Ex/Em = 360/460 nm.

2. Flow Cytometry Analysis

(1). Prepare the Click-iT working solution according to the table below.

Note: Add the components in the order listed in the table, otherwise the reaction will not yield optimal results. The prepared Click-iT working solution must be used within 15 min.

Component	Number of Samples						
	1	2	4	5	10	25	50
Deionized Water	189.5 µL	379 µL	758 µL	947.5 µL	1,895 µL	4,737.5 µL	9,475 µL
Reaction Buffer (10×)	25 µL	50 µL	100 µL	125 µL	250 µL	625 µL	1,250 µL
Copper Reagent	10 µL	20 µL	40 µL	50 µL	100 µL	250 µL	500 µL
AbFluor 545 Azide	0.5 µL	1 µL	2 µL	2.5 µL	5 µL	12.5 µL	25 µL
Reducing Agent (10×)	25 µL	50 µL	100 µL	125 µL	250 µL	625 µL	1,250 µL
Total Volume	250 µL	500 µL	1,000 µL	1,250 µL	2,500 µL	6,250 µL	12,500 µL

(2). Centrifuge at 800 g for 5 min, remove the permeabilization buffer, collect the cells, and add 250 µL of Click-iT working solution. Incubate at room temperature for 30 min, protected from light.

(3). Centrifuge at 800 g for 5 min, remove the Click-iT working solution, and wash the cells with 500 µL of BSA Wash Solution (1×) for 5 min. Repeat this wash step 3 times.

(4). Optional step: Centrifuge at 800 g for 5 min, remove the wash solution, and perform nuclear staining (1× Hoechst 33342, incubate at room temperature for 10 min, protected from light) or antibody labeling.

Important tip: Samples must be protected from light during all incubation steps. If no additional staining is required, proceed directly to imaging and analysis after incubation.

(5). Analyze the cells by flow cytometry.

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.

FAQ

1. Can this kit be used to stain mesenchymal stem cells and then perform in vivo transplantation?

A: It is recommended that the staining effect be first evaluated in vitro culture prior to transplantation experiments, and that color development and observation be completed within one week.

2. Can this kit be used in combination with immunofluorescence (IF) for dual staining? If so, what is the recommended sequence of the two procedures?

A: This product can be used for double staining with immunofluorescence (IF). It is recommended that EdU labeling be performed first, followed by IF staining.

3. How should the EdU incubation time be adjusted?

A: The duration of incubation is dependent on the rate of cell growth, and it is generally appropriate to incubate for a period equivalent to approximately 10% of the cell cycle. When the incubation time is less than 45 min, an increase in EdU concentration is recommended; when the incubation time exceeds 20 hours, a decrease in EdU concentration is advisable.

Cell Type	Cell Cycle	Incubating Time
Mammalian cells, including HeLa, 3T3, HEK293, and others	18-25 h	2 h
Human Embryonic Cell	30 min	5 min
Yeast Cell	3 h	20 min
Proliferating nerve cells	5 d	1 d

4. The background signal is too high for fluorescence imaging.

A:

① Optimize the washing steps, especially after the staining is completed, ensure that the staining working solution is completely removed and the cells are fully washed to remove the residual staining solution and avoid the situation of excessive background signal during fluorescence imaging.

② Optimize and adjust the parameters of fluorescence microscope image acquisition to avoid overexposure.

5. The signal of fluorescence imaging is relatively weak.

A:

① Insufficient EdU concentration or labeling time leads to insufficient labeling of proliferating cells, so the EdU concentration and labeling time need to be adjusted.

② The parameters of fluorescence microscope image acquisition should be optimized to improve the gain of fluorescence microscope or exposure time, and over-exposure should be avoided.

Recommended Products

Catalog No.	Product Name
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA2011	One-step TUNEL Apoptosis Assay Kit (Red Fluorescence)
KTA2020	Cell Cycle Staining Kit
KTA2030	EdU Cell Proliferation Imaging/Flow Cytometry Assay Kit (Green Fluorescence)

Disclaimer

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