Click-iT EdU-647 Cell Proliferation Assay Kit 100T

AO-03-G1604-100T

Product Information

Product Name	Cat. No	Spec.
Click-iT EdU-647 Cell Proliferation Assay Kit	G1604	100 T

Product Description/Introduction

Analyzing cell proliferation ability is a common and important evaluation method in life sciences. It can judge the influence of certain genes, drugs, etc. on cells cultured in vitro, or analyze the growth and renewal ability of tissue cells under different conditions or stimulation. At present, there are many methods to detect cell proliferation. Most of them use some metabolic enzymes produced by cells to indirectly assess cell proliferation activity (such as CCK-8 method, MTT method, etc.), but some drugs or the state of the cell itself will have a certain impact on the results of the assessment. Direct detection of DNA synthesis in cells to determine cell proliferation is recognized as the most accurate and effective detection method. However, both the original radiolabeled nucleoside incorporation method and the subsequent improvement of the BrdU method based on antibody detection have their own limitations.

EdU (5-Ethynyl-2'-deoxyuridine, 5-ethynyl-2'-deoxyuridine) is a thymidine analogue containing an acetylene group, when injected into animals or incubating cells cultured in vitro, these small molecules can quickly diffuse to various organs and tissues, and infiltrate into the cells, and can replace thymidine (T) into newly synthesized DNA during cell proliferation. The acetylene group in the EdU molecule can react with the fluorescently iF488 labeled azide compound probe to form a stable triazole ring under the catalysis of copper ions, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe. Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limiting factors such as radioactive contamination; compared with the BrdU detection method, the EdU detection method does not require DNA denaturation or antigen-antibody reaction, which greatly reduces the complexity of the experiment and also make the experiment more time-saving, more sensitive, more stable and more accurate.

This kit can be used to detect cell proliferation in cultured cells or animal tissues. The fluorescent probe in this kit is pink (Far Infrared) fluorescence, the maximum excitation wavelength is 656 nm, and the maximum emission wavelength is 670 nm. After the proliferating cells are labeled, the cell nucleus will show bright pink (Far Infrared) fluorescence, and the cell nucleus will be jointly labeled with the matching conventional nuclear dye (This kit provides Hoechst 33342 cell nuclear dye), you can use fluorescence microscope, laser confocal microscope and other instruments to directly observe cell proliferation; you can also use flow cytometry to detect the fluorescence intensity of cultured cells in vitro, and then determine the cell cycle based on the fluorescence intensity DNA replication activity in mid-S phase.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C in the dark. Catalyst (Reagent A) and Reaction Buffer can be stored at 4°C; Valid for 12 months.

Product Contents

Component Number	Component	G1604-100T
G1604-1	EdU Stock Solution (10 mM)	100 μL
G1604-2	Catalyst (Reagent A)	120 μL
G1604-3	Fluorescent Stain iF647（Reagent B）	50 μL
G1604-4	Catalytic Additive (Reagent C)	2×100 mg（powder）
G1604-5	Reaction Buffer	20 mL
G1604-6	Hoechst 33342 Stain	30 μL
Manual		One copy

Note: The above reaction times are corresponding to 96-well plate detection.

Before starting

1. Serum-containing cell culture medium;

2. Permeabilization buffer: buffer containing 0.2-0.5% Triton X-100 (recommended G1204);

3. Fixative: 4% paraformaldehyde (recommended G1101) or other similar reagents;

4. PBS buffer (recommended G4202);

5. Ultrapure water;

6. Animal modeling and tissue section related reagents (animal tissue cell proliferation detection).

Assay Protocol / Procedures :

1. Pretreatment of cultured cells in vitro:

1.1. Plant the cells evenly in the cell culture plate at a certain density (the planting density is determined by factors such as cell size, growth speed, etc.). After the cells adhere to the wall or return to a normal state, perform corresponding drug stimulation and other treatments. (For suspension cells, please follow the normal operation method of suspension cells. The whole experiment needs to add centrifugation and other steps).

1.2. The catalytic additive (Reagent C) was centrifuged at low speed, 100 mg was taken and dissolved by adding 1 mL of ultrapure water and dispensed and stored at -20°C, the remaining powder was kept as reserve.

2. In vitro cellular EdU labeling, fixation and permeabilization:

2.1. Prepare 2×EdU incubation working solution: add 2 μL EdU storage solution (10 mM) to every 1 mL of complete cell culture medium, which is 20 μM 2×EdU incubation working solution, and put it in the incubator to preheat (the recommend EdU working concentration is 10 μM for preliminary experiments);

2.2. In the half-changing mode, aspirate half of the original cell culture medium in the culture plate, and add an equal volume of preheated 2×EdU incubation working solution, and incubate for a certain period of time (the duration of the incubation generally depends on the growth cycle of the corresponding cells, which usually accounts for about 10% of the cell cycle. For mostly adherent and fast-growing cells, incubation for about 2 h is recommended. For specific cases, it needs to be adjusted with the cell characteristics, the actual

situation after treatment, etc. If a longer incubation time is required, the EdU working concentration can be appropriately reduced; for a shorter time, the EdU concentration can be appropriately increased);

2.3. After EdU incubation, wash with PBS buffer for 1-2 times, add fixing fluid to cover the cells, and fix at room temperature for 15 minutes (if flow cytometry is required, the adherent cells should be digested and resuspended before this step fix, follow the suspension cell processing method); Wash 2-3 times with PBS buffer, 3-5 min each time;

2.4. Remove the PBS buffer, add permeabilization solution to cover the cells, and incubate at room temperature for 15 minutes;

2.5. Remove the permeabilization solution, wash 1-2 times with PBS buffer, 3-5 min each time. Then go to step 4.

3. Animals EdU injection modeling as well as tissue section processing:

3.1. According to experimental requirements, one or more EdU injections are used to model animals by intraperitoneal injection, intramuscular injection, subcutaneous injection, tail vein injection, etc. Generally, the ratio of EdU dosage to animal body weight is 5 mg/kg, the actual injection dose depends on the research content and animal conditions. The EdU storage solution provided in this kit is mainly used for in vitro cell EdU labeling. If you need to model an animal with EdU, you can order the EdU reagent separately (Cat. No.: G5059);

3.2. Epithelial cells such as the small intestine proliferate quickly, while brain cells proliferate slowly. The faster-growing tissues usually take less than 12 hours for labeling, while those slower-growing tissues may take several days for labeling.The optimal labeling time was determined according to the specific experiment. Due to the rapid proliferation of intestinal epithelial tissue, such tissue was recommended as a reference for labeling.

3.3. After the animal is killed according to the specified standards, the tissues needed are taken out and frozen sections or paraffin sections are made according to the conventional procedures ：

a. For frozen sections: return the sections to room temperature, add an appropriate amount of Fixing fluid, and fix at room temperature for 15 minutes. Remove the Fixing fluid and wash 3 times with PBS buffer for 3-5 min each; remove the PBS buffer and cover the tissue with an appropriate amount of permeabilization solution and incubate at room temperature for 10-15 min; remove the permeabilization solution and wash with PBS buffer 1- 2 times, 3-5 min each time. Then go to step 4.

b. For paraffin sections: Deparaffinize and rehydrate the sections, and wash with PBS for 5 min. Remove the PBS buffer, add permeabilization solution to cover the cells or tissues, and incubate at room temperature for 15 min; Then wash with PBS buffer for 1-2 times, each time for 3-5 min. Then go to step 4.

4. EdU click reaction:

4.1. During cell or tissue fixation and perforation, preparation of reaction solution: mix the reagents according to the following ratio, the volume of preparation can be increased or decreased in proportion to the number of samples.

Component	Volume (for cell)	Volume (for Histological section)
reaction buffer	935 μL	928 μL
Reagent A	10 μL	10 μL
Reagent B (iF647 dye)	5 μL	12 μL
Reagent C	50 μL	50 μL
total capacity	1000 μL	1000 μL

4.2. Remove the PBS buffer from the cells or sections, add the reaction solution, shake gently to ensure that the reaction solution covers all the cells or tissues, and incubate for 30 min at room temperature in the dark;

4.3. Remove the reaction solution, wash 2-3 times with PBS buffer, 3-5 min each time (If there is no other special requirement, the fluorescence intensity can be detected by flow cytometry or the fluorescence effect can be detected by other instruments).

5. Nuclear stain(optional):

5.1. Dilute Hoechst 33342 staining solution with PBS buffer at a ratio of 1:500-1000, add to the sample to cover the cells, and incubate for 5 min;

5.2. Remove Hoechst 33342 staining solution, wash 2-3 times with PBS buffer, 3-5 min each time.

6. Imaging and detection analysis:

Use fluorescence microscope or confocal microscope to detect processed cells or tissue section samples, and analyze the proportion of proliferating cells. Alternatively, cells cultured in vitro can be collected and the fluorescence intensity can be measured by flow cytometry (it is recommended to use cell samples not labeled with EdU as a negative control for the flow cytometry assay and to choose the appropriate voltage), and based on the fluorescence intensity, the DNA replication activity of the S-phase in the cell cycle can be determined. The fluorescent dye iF647 (Reagent B) in this kit corresponds to the spectral characterization of Ex/Em: 656 nm/670 nm (pink); Hoechst 33342 staining solution corresponds to the spectral characterization of Ex/Em: 346 nm/460 nm (blue).

Note:

1. For cultured cells, the specific EdU concentration and incubation time can be adjusted appropriately depending on the sample and research purpose.

2. Some tissue cells proliferate slowly. In order to avoid poor modeling effect, it is recommended to select tissue samples with fast proliferation as reference samples (such as intestinal tissue).

3. If the background color is too dark, it may be caused by insufficient washing, a long time fixed, and residual fixative, etc.in the experiment.

4. Reagent C (EdU catalytic addition reagent) is easy to oxidize. Try to avoid prolonged exposure to the air. After being prepared as an aqueous solution, it is recommended to store in aliquot; Tested, such as EdU catalytic additive reagent color changes slightly, click reaction catalytic system is still able to proceed normally. if reagent C appears brown, it indicates that the component has expired, so please discard it.

5. For your health and safety, please wear lab coats and gloves during operation.

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