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MCE® MedChemExpress

VF 647 Click-iT EdU Universal Cell Proliferation Detection Kit

1 Contents

Components	HY-K1084-100T	HY-K1084-500T	Storage Conditions After Opening
10 mM EdU	200 μL	1 mL	-20°C, 1 year
VF 647 Azide	50 μL	250 μL	-20°C, Protect from light, 1 year
10 × Click-iT Reaction Buffer	1 mL	5 mL	2-8°C,1 year
$CuSO_4$	500 μL	2 × 1.25 mL	2-8°C, 1 year
Click-iT Additive	30 mg	150 mg	2-8°C, 1 year
Hoechst 33342	25 μL	125 μL	2-8°C, 1 year

2 Introduction

The ability of cells proliferate is a crucial for assessing cell activity, determining genotoxicity, and evaluating anti-cancer drugs. The most accurate method of is to directly measuring DNA synthesis. The traditional BrdU (5-Bromo-2'-deoxyridine) method is step-intersive and requires the use of BrdU antibodies, which have many influencing factors and poor stability. The EdU (5-Ethynyl-2'-deoxyridine) method is a new and upgraded method. It only requires paraformaldehyde fixation and Triton X-100 penetration promotion to allow the detection reagent to enter the cell. A small amount of azide dye can quickly and effectively mark the integrated EdU by Click Chemistry.

MedChemExpress VF 647 Click-iT EdU Universal Cell Proliferation Detection Kit contains EdU and VF 647 Azide dye, along with the catalysts and buffers required for click chemistry. It can be used for simple, rapid, efficient and sensitive cell proliferation assays.

Hoechst 33342 is also provided in the kit for cell cycle analysis. This kit is suitable for fluorescence microscopy, laser confocal microscopy or flow cytometry detection. 100T refers to detect 100 samples for 96-well plates.

3 General Protocol

Materials required but not provided

- Cell fixative: 4% paraformaldehyde
- Penetrant solution: 0.5% Triton X-100
- 2 mg/mL glycine solution
- Washing solution: 3% BSA
- Preservation solution: 1% BSA

Note: Except for glycine, the above reagents are prepared with PBS (pH 7.2-7.6).

Pre-Experiment

- Preparation of 1 × Click-iT Action Buffer: dilute 10× Click-iT Reaction Buffer to 1 × with ddH_2O.
- Preparation of 5 × Click-iT Additive Solution: add 300 μL ddH_2O to 30 mg of Click-iT Additive in separate tubes (final concentration 100 mg/mL), mix until completely dissolved. Store at -20°C for 1 year. If the solution develops a brown color, it has degraded and should be discarded.
  Note: Different sizes of Click-iT Additive are dissolved in this ratio with ddH_2O and prepared to 5 ×.
- Preparation of 1 × Click-iT Additive Solution: dilute 5 × Click-iT Additive Solution to 1 × with ddH_2O, the solution should be prepared and used immediately.

Protocol

Note: This reference needle is based on a 96-well plate.

1. Cell culture

Take logarithmic growth phase cells, inoculate in 96-well plates at 4×10^3 - 1×10^5 cells (the number and density of cells can be adjusted according to cell size, growth rate, and specific requirements of the experimental treatments), and them to the normal growth phase.

2. Drug treatment

Various drug treatments or other stimulation treatments, etc. are performed according to the experimental needs.

3. EdU marking

1) Dilute the EdU solution with complete cell culture medium to a certain appropriate concentration, add it to cells and mix well; set up a negative control group without EdU treatment.

Note: For adherent cells such as A594, HeLa, and NIH/3T3, the final concentration of EdU is 10 μM. Growth medium, cell densities, cell type variations, and other factors may influence labeling. In initial experiments, we recommend testing a range of EdU concentrations to determine the optimal concentration for your cell type and experimental conditions. If you are currently using a BrdU-based assay for cell proliferation, a similar concentration to BrdU is a good concentration for EdU.

2) Incubate for 2 hours in cell incubator, and remove the media.

Note: The optimal incubation time depends on the growth rate of the cells, and usually the preferred incubation time is about 10% of the cell cycle. For common mammalian cells such as HeLa, 3T3, HEK293, etc., the cell cycle is about 18 - 25 hours, and the incubation time is generally around 2 hours. The cell cycle of human embryonic cells is about 30 minutes, and the incubation time is 5 minutes. The cell cycle of yeast cells is about 3 hours, and the incubation time is 20 minutes. The cell cycle of nerve cells is about 5 days, and the incubation time is 24 hours. The concentration of EdU is related to the incubation time, it is better to use a high concentration, e.g., 10 - 50 μM for short incubation time (< 2 h), and a low concentration, e.g., 1 - 10 μM for long incubation time (> 24 h).

4. Cell fixation and permeabilization

Note: For experiments requiring surface antigen labeling, consider washing cells twice with washing solution after completion of EdU incubation and before cell immobilization.

1) Wash cells twice with PBS for 3 - 5 minutes each time to remove EdU residues that are not mixed with DNA. Reduce the number of washes for poorly adherent cells.

Note: Centrifugation conditions can be set based on specific cell culture experiences.

2) Add 50μL of cell fixative to each well, incubate at room temperature for 30 minutes, and remove the fixative.

Note: Low concentration of paraformaldehyde fixative is beneficial for retention cell structure, but other methods of cell fixation can also be used. The cell sedimentation coefficient changes after the fixation treatment, the centrifugal force needs to be adjusted according to the cell conditions.

3) Add 50 μL of Glycine solution to each well, incubate at room temperature for 5 minutes, and remove the Glycine solution.

Note: This step is to neutralize the excessive aldehyde groups and ensure the dyeing reaction system. It can be omitted when using non-aldehyde fixatives for cell fixation.

4) Add 100 μL of washing solution to each well to wash the cells twice, each time for 3 - 5 minutes, and remove the washing solution.

5) Add 100 μL of penetrant solution to each well, incubate at room temperature for 10 minutes, and remove the permeabilization buffer.

Note: If necessary, the permeabilization time can be extended to increase cell membrane permeability.

5. EdU detection

Note: This reference procedure is for 96-well plates with working solution of 100 μL each well, and users can adjust the amount according to sample conditions.

1) Preparing Click-iT working solution according to table below.

Note: Click-iT working solution within 15 minutes of preparation.

Click-iT working solution

Reaction components	Take the sample size of 10 wells as an example
1 × Click-iT Reaction Buffer	855 μL
$CuSO_4$	40 μL
VF 647 Azide	5 μL
1 × Click-iT Additive Solution	100 μL
Total Volume	1000 μL

2) Add 100 μL of washing solution to each well to wash the cells twice, and remove the washing solution.

3) Add 100μL of Click-iT working solution to each well of permeabilized cells and mix well to insure that the working solution is distributed evenly over the coverslip. Incubate the plate for 30 minutes at room temperature, protect from light, and remove the working solution.

4) Add 100 μL of washing solution to each well to wash the cells twice and remove washing solution, and add 100 μL of preservation solution to each well to keeps cells moist. If there is no other special requirement, proceed to Imaging and analysis.

6. DNA re-staining or intracellular antigen labeling (optional step)

DNA re-staining

1) Dilute the Hoechst 33342 solution 1:2000 in PBS to obtain a 1 × Hoechst 33342 solution, and store at 2 - 8°C, protect from light.

2) Add 100 μL of Hoechst 33342 solution (1 ×) to each well, incubate for 15 - 30 minutes at room temperature, protect from light. Remove the Hoechst 33342 solution.

3) Wash each well 1 - 3 times with 100 μL of PBS. Remove the PBS.

Intracellular antigen labeling

1) Add antibody and mix well.

2) Incubate the antibodies at appropriate temperature, protect from light.

7. Imaging and analysis

1) Fluorescence microscopy or flow cytometry is recommended immediately after staining; if conditions are limited, keep it in humidified storage at 4°C, and protect from light to wait for the test no more than 3 days. For cell sides or smears, it can be sealed with an anti-fluorescence quenching sealing machine and stored in dark at 4°C to improve the preservation effect.

2) Hoechst re-staining can be used to observe the staining of proliferating cells. Hoechst 33342 is blue fluorescent with a maximum excitation wavelength of 350 nm and a maximum emission wavelength of 461 nm. The VF 647 Azide is red fluorescent with a maximum excitation wavelength of 650 nm and a maximum emission wavelength of 670 nm.

3) The recommended number of cells for flow cytometry testing should be in the millions. If the cell yield is too low (just to 10000), the number of washes in step 5 (3) can be reduced.

4) Due to the sensitivity of flow cytometry, the amont of VF 647 Azide can be adjusted appropriately according to cell type and actual staining sconditions.

4 Storage

Store at - 20°C, 3 years.

Protect from light and avoid repeat freeze-thaw cycles.

5 Precautions

1.Please centrifuge before use.

2.Please protect from light during experimental operations to slow down quenching of fluorescence.

3.Click-iT Additive should be prepared as a solution and be stored at -20°C. If a white substance precipitates after dissolution, turn the solution upside down several times and use it fleshly.

4.Reaction and detection prior to the Click reaction is required for fluorescent proteins such as GFP, TC-FLAsH and TC-ReAsH reagents, as this product affects the fluorescence of fluorescent proteins. Since Phalloidin is not compatible with the Click reaction, Tubulin-Tracker Red (HY-131010) is recommended for the detection of cellular microtubules.

5.This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

6.For your safety and health, please wear a lab coat and disposable gloves to operate.