SF594 EdU Flow Cytometry Kit (Red Fluorescence)

Cat: CA1174-B
Size: 20T/50T
Storage: -20℃, avoid light.

Product introduction:

Cell proliferation detection is a basic experimental means to evaluate the health degree of cells, genotoxicity and the effect of anti-tumor drugs. The most accurate method for measuring cell proliferation is the BrdU method. The EdU test kit is a revolutionary breakthrough from the BrdU method. EdU (5-acetylidene-2 '-deoxyuridine) is a pyrimidine analogue that is integrated into the DNA double strand during DNA synthesis. The EdU assay is based on the "Click-iT" reaction, a copper-catalyzed reaction of an azide compound with an alkyne to form a covalent bond.

In this kit, EdU contains alkynes, and SF488/555/594/647A Azide dye contains azide compounds. The Click-iT method of EdU labeling proliferates quickly and efficiently and is easy to use. The BrdU method requires DNA denaturation (e.g., acid denaturation, thermal denaturation, or digestion with DNase) to expose BrdU and facilitate BrdU antibody binding; The EdU method requires only paraformaldehyde fixation and Triton X-100 osmotic induction to allow the detection reagent to enter the cell, and only a small amount of azide dye is required to mark the integrated EdU very effectively.

This kit contains all components required for EdU assay and can be used for proliferation detection of cultured cells in vitro.

Product composition:

Components	CA1174-B -20T	CA1174-B -50T	Storage conditions after opening	Stability
A. 10mM EdU	0.4mL	1mL	- 20 ℃	Store at specified temperature after opening for one year
B. SF488/555/594/647A Azide	100μL	250μL	-20 ℃, away from light	
C. 10 x Click-iT EdU reaction buffer	2×1mL	5mL	2-8 ℃	
D. $CuSO_4$	0.8mL	2×1mL	2-8 ℃	
E. Click-iT EdU buffer addition	60mg	150mg	2-8 ℃	

The above reaction times are for cells cultured in 6-well plates, and the specific dosage of different containers can be referred to Attached Table 1.

Fluorescence spectral data: SF488 Azide: 495/519nm; SF555 Azide: 555/565nm; SF594 Azide: 590/617 nm; SF647A Azide: 650/670nm.

Protocols:

Experimental materials (bring your own)

1. 10mM PBS, pH 7.2-7.6

2. 4% paraformaldehyde fixing solution
3. Osmotic enhancer (0.5% Triton X-100 in PBS)
4. 1% BSA in PBS, pH 7.2-7.6
5. ddH_2O
6. 2mg/mL glycine solution (in ddH_2O)
7. 96/24/12/6 well culture plate or dish

Experimental steps (for reference only) :

1. Cell culture

1×10^5~3×10^6 cells per well were inoculated in the 6-well plate.

2. Drug treatment

Perform various drug treatments according to the needs of the experiment.

3. EdU labeled cells

(1) Dilute EdU solution (Component A) to an appropriate concentration with cell complete culture medium, and then add it into the cells and mix well; A negative control group was set up without EdU treatment.

Note: The labelled EdU concentration needs to be adjusted according to cell type, and an initial concentration of 10μM is recommended. For pre-experiments, it is recommended to set the EdU concentration gradient, which can be seen in Schedules 2 and 3.

(2) Incubate in cell incubators for 2 h. EdU Incubation time of cells can be used directly as an indicator of cell DNA synthesis, the choice of time point and the time of incubation depends on the cell growth rate. Pulsed labeling of cells by short EdU incubation can be used to study cell cycle dynamics.

Note: The optimal incubation time is related to the cell cycle, and the incubation time of 2h is available for most tumor cell lines, as shown in Table 2. EdU concentration is related to incubation time, and high concentration should be used for short incubation time (<2h), such as: 10~50μM; Long-term incubation (>24 h) should use a low concentration, such as: 1~10μM; Also refer to Schedule 3.

4. Cell fixation and osmosis promotion

Note: For experiments requiring cell surface antigen labeling, consider washing cells with 1% BSA detergent solution twice after EdU incubation, before cell fixation and osmotic promotion.

(1) After incubation, cells were collected, cells were cleaned with 1mL PBS added to each tube, centrifuged at 1000rpm for 5 min, and supernatant was sucked up to remove EdU residue that was not incorporated DNA.

(2) The cells were re-suspended by adding 1mL of 4% paraformaldehyde fixing solution per tube.

(3) Incubate at room temperature for 20 min, centrifuge at 1000 rpm for 5 min, then discard the supernatant.

(4) Add 1mL of 2mg/mL of glycine to each tube and incubate for 5min, neutralize the residual

(5) fixing solution, centrifuge at 1000rpm for 5min, then discard the supernatant, add 1mL of PBS to each tube and clean once, centrifuge at 1000rpm for 5min, then discard the supernatant.

(6) 1mL of 0.5%Triton X-100 soaking-promoting solution resuspension cells were added into each tube and incubated at room temperature for 10min.

5. EdU test

Note: For the 6-hole plate sample can refer to each hole 1mL working fluid to carry out, the user can adjust the dosage according to their own sample situation.

(1) Configure 1×Click-iT EdU reaction buffer (Component C) : Dilute component C by 10 times with ddH_2O.

(2) Configure 5×Click-iT EdU buffer additive (Component E) : add 300μL of ddH_2O to 30mg of Group E tube (final concentration 100mg/mL), mix until completely dissolved. After use, the remaining storage solution is stored at -20℃ and can be stored for one year. Once the solution turns brown, it indicates that the active component is degraded and can not be used again.

Note: Components E of different specifications are dissolved with ddH_2O in this ratio and prepared into 5× storage liquid for use.

(3) Prepare 1×Click-iT EdU buffer addition: Dilute the 5×Click-iT EdU buffer addition storage solution to 1× with ddH_2O, and the solution should be ready for use.

(4) Prepare the Click-iT working solution according to Table 1.

Table 1: Click-iT working fluid

Reaction Components	Liquid volume required for a single reaction
1 x Click-iT EdU reaction buffer	875μL
$CuSO_4$ (Component D)	20μL
SF488/555/594/647A Azide (Component B)	5μL
1 x Click-iT EdU buffer addition	100μL
Total volume	1mL

(5) Centrifuge at 1000rpm for 5min, then discard the supernatant and remove the osmotic enhant. Add 1mL of 1% BSA washing solution to each tube for 2 times, and centrifuge at 1000rpm for 5min, then discard the supernatant.

(6) Add 1mL Click-iT working liquid into each tube and mix well.

(7) Incubate at room temperature without light for 30min.

(8) Centrifuge at 1000rpm for 5min, then discard the staining reaction solution, add 1%BSA to each tube to wash the cells twice, centrifuge at 1000rpm for 5min, then discard the supernant, then re-suspend the cells with 1mL 1%BSA (the volume of the solution of the suspended cells can be adjusted according to the number of cells), and then detect by flow cytometry.

Note: If other markers need to be detected, please refer to Step 4.

6. Intracellular antigen labeling (optional step)

(1) Add the antibody working solution and mix well.

(2) Incubate the antibody at the appropriate temperature and time under the condition of avoiding

light.

7. Flow detection and analysis:

(1) It is recommended to carry out flow detection immediately after dyeing; If the conditions are limited, please avoid light 4℃ moist store to be measured, but should not exceed 3 days.

(2) It is recommended that the number of detected cells should reach millions as far as possible. If the number of detected cells is small, the number of detected cells can be adjusted to 100,000 to start the experiment. For the case that the cell yield is too small (just to 10,000 cells), it may not be conducive to doing flow diagram, which can be appropriately reduced in step 5 (8) cleaning times.

Not:

1. Please centrifuge the product to the bottom of the tube instantaneously before use, and then conduct follow-up experiment.
2. The dye has the quenching problem, please try to avoid light during the experimental operation, in order to slow down the fluorescence quenching.
3. Click-iT EdU buffer additive solution is best prepared and used to ensure the best results.
4. For your safety and health, please wear a lab coat and wear disposable gloves.