EdU Cell Proliferation Detection Kit (AF594)

E1373492

Storage: -20℃. Protect from light.

Introduction

Cell proliferation assays are widely used in the evaluation of cell viability, genotoxicity, and the efficacy of antitumor drugs. Direct detection of DNA synthesis in cells is considered the most accurate method for assessing cell proliferation. EdU (5-ethynyl-2′-deoxyuridine) is a novel thymidine (thymine deoxyribonucleoside) analogue. During DNA synthesis, EdU can be incorporated into newly synthesized DNA in place of thymidine. The ethynyl group on EdU can undergo a covalent reaction with fluorescently labeled small-molecule azide probes (such as Azide Alexa Fluor 488, Azide Alexa Fluor 555, Azide Alexa Fluor 594, Azide Alexa Fluor 647, etc.) via Cu(I)-catalyzed click chemistry, forming a stable triazole ring. This reaction is highly efficient and is referred to as the Click reaction. Through this process, newly synthesized DNA is labeled with the corresponding fluorescent probes, enabling the detection of proliferating cells using appropriate fluorescence detection equipment.

Kit Contents

E1373492	Component	50 T	100 T	200 T	Storage conditions	Quantity Per Test
E1373492A	EdU(10 mM)	100 μL	200 μL	400 μL	-20℃.Store in the dark.	2 μL per 1.0-2.0x10^6 cells
E1373492B	AF594 azide	250 μL	500 μL	1000 μL	-20℃.Store in the dark.	5 μL per 1.0-2.0x10^6 cells
E1373492C	Click Reaction Buffer	12 mL	24 mL	48 mL	-20℃.Store in the dark.	240 μL per 1.0-2.0x10^6 cells
E1373492D	$CuSO_4$	250 μL	500 μL	1000 μL	-20℃.	5 μL per 1.0-2.0x10^6 cells
E1373492E	Click Additive	991 mg	1982 mg	3964 mg	-20℃.Store in the dark.	250 μL per 1.0-2.0x10^6 cells
E1373492F	DAPI Staining Solution (1000×)	25 μL	50 μL	100 μL	-20℃.Store in the dark.	0.5 μL per 1.0-2.0x10^6 cells

Note: The recommended number of cells to stain per test is 1.0-2.0x10^6 cells. The amount of Click reaction solution can be adjusted according to the experimental samples.

Instruction for use

1. Preparation

1) Preparation of Click Additive Solution:

For a 50-test kit: Add 12.5 mL of pre-chilled deionized water to the tube. Mix thoroughly until completely dissolved to obtain the Click Additive Solution. For a 100-test kit: Add 25 mL of pre-chilled deionized water to the tube. Mix thoroughly until completely dissolved to obtain the Click Additive Solution. For a 200-test kit: Add 50 mL of pre-chilled deionized water to the tube. Mix thoroughly until completely dissolved to obtain the Click Additive Solution. After preparation, aliquot the solution as needed and store at -20°C. If a white precipitate forms after dissolution, invert the tube repeatedly until it is fully dissolved before use. If the solution turns brown, it indicates degradation of the active component; discard it.

2) Upon initial dissolution of the Click Reaction Buffer, aliquot it according to the number of samples per experiment and store at -20°C.

2. EdU Labeling of Cells

It is recommended to use a final EdU concentration of 10 μM (1×). A 1:500 dilution of EdU (10 mM) in cell culture medium yields a 2× EdU working solution (20 μM). Mix an equal volume of pre-warmed (37°C) 2× EdU working solution (20 μM) with the cell suspension to achieve a final 1× EdU concentration. Incubate in a 37°C, 5% CO_2 incubator. Factors such as cell culture medium, cell density, cell type, and other experimental conditions may affect labeling efficiency. Therefore, the optimal EdU concentration and labeling duration must be empirically determined based on the cell type under investigation.

3. Fixation and Permeabilization

1) Harvest cells and centrifuge at 300 ×g for 5 min. Wash cells twice with PBS containing 2% FBS.

2) Fix cells with 4% paraformaldehyde solution. Mix thoroughly and incubate for 15 min at room temperature protected from light.

3) Collect cells and centrifuge at 300 × g for 5 min. Wash cells twice.

4) Resuspend cells in PBS containing 0.3% Triton X-100. Mix well and incubate for 15 min at room temperature.

5) Centrifuge at 300 × g for 5 min and wash cells twice.

4. Fluorescent Labeling

1) This protocol is based on a 500 μL reaction system per 2×10^6 cells. The volume of the Click reaction mixture can be adjusted according to the experimental sample size.

2) Centrifuge the cells at 300×g for 5 minutes. Add 500 μL of Click reaction mixture per sample, mix gently, and incubate for 30 minutes at room temperature protected from light.

3) After the reaction, wash the cells twice with PBS containing 2% FBS.

4) Dilute the DAPI Staining Solution (1000×) to 1× using PBS containing 2% FBS. Add 250 μL

of the diluted DAPI solution to each sample and incubate for 5 minutes at room temperature.

5) Add an additional 250 μL of PBS containing 2% FBS, mix gently, and proceed to detection using an appropriate flow cytometry instrument.

Component	Number of samples						
	1	2	4	5	10	25	50
Click Reaction Buffer	240 μL	480 μL	960 μL	1200 μL	2400 μL	6000 μL	12000 μL
$CuSO_4$	5 μL	10 μL	20 μL	25 μL	50 μL	125 μL	250 μL
Azide 594	5 μL	10 μL	20 μL	25 μL	50 μL	125 μL	250 μL
Click Additive Solution	250 μL	500 μL	1000 μL	1250 μL	2500 μL	6250 μL	12500 μL
Total volume	500 μL	1000 μL	2000 μL	2500 μL	5000 μL	12500 μL	25000 μL

Matters needing attention

1. Strictly adhere to the component order and volumes specified in the table above when preparing the Click reaction mixture, as deviations may affect experimental results.
2. The Click reaction mixture must be used within 15 minutes of preparation.
3. To avoid fluorescence quenching, perform detection as soon as possible after sample preparation.