TransDetect® EdU Flow Cytometry Kit-488 Fluorophore

Please read the datasheet carefully prior to use.

Cat. No. FU201

Storage: at 2-8°C in the dark for one year

Description

The kit is designed for detecting the ability of cell proliferation with the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU). In the process of DNA replication, EdU can be inserted into the newly synthesized DNA double-stranded structure, and then EdU can be labeled by fluorescent groups by click reaction. Flow cytometry method is used to detect the DNA replication activity of the S phase according to the fluorescence intensity, so as to detect the cell proliferation ability. The maximum excitation wavelength and emission wavelength of 488 Fluorophore are 494 nm and 520 nm respectively.

Compared with traditional BrdU assay methods, this kit does not require additional steps such as antibody labeling, is easy to operate, has high sensitivity and specificity. It is suitable for drug screening, cell proliferation assay, cytotoxicity assay and other experiments.

Features

- No need to use antibodies, simple operation.
- High sensitivity and specificity.
- Wide application range.

Kit Contents

Component	50 rxns
EdU (10 mM)	1 ml
488 Fluorophore-FC	250 µl
Catalyst Solution (CS)	250 µl
EdU Buffer Additive (EBA)	2×200 mg
EdU Fixation Buffer (EFB)	5 ml
EdU Permeabilization/Wash Buffer (10×) (10×EPWB)	50 ml

Procedures

Self-prepared

Product Name	Catalog
PBS (1×)	TransGen, Cat. FG701-01
BSA	Sigma, Cat. A1933

EdU Label

Take 6-well cell culture plates as an example

1.1 0.5-1×10^6 cells are seeded in 6-well cell culture plate and cultured overnight or treated with drugs.

1.2 2×EdU working solution preparation: add 4 µl EdU (10mM) into per 1 ml cell complete medium to obtain 2×EdU working solution with a concentration of 40 µM.

1.3 The 2×EdU working solution with the same volume as the original medium is added to the 6-well cell culture plate, and the final concentration of EdU in the culture medium is 20 µM. Incubate at 37°C and 5% CO_2 incubator. It is not recommended to remove the original medium completely, as this may affect cell proliferation. At the same time, set up 1 control group without EdU (replace 2×EdU with an equal volume of complete medium) as dye background analysis of flow cytometry data.

The optimal incubation time of cells is related to the cell growth cycle, which is generally 1/10 to 1/5 of the cell cycle. Most cell lines can be incubated for 2 hours. It is recommended to set the gradient for the first test to explore the optimal incubation time. The recommended incubation time for different cell types is shown in the table below:

Cell Type	Cell	Incubation Time (h)
Tumor Cells	A549	2
	NS-1	2
	HeLa	2
Primary Cells	HUVEC	2
Nerve Cells	SH-SY5Y	2
Human Embryonic Stem Cells	H9	24
Other Cells	NIH/3T3	1.5
	MARC145	1.5

1.4 Tryptic Digestion. Centrifuge at 200×g for 5 min. Discard the supernatant to collect the cells. Suspension cells can be collected directly by centrifugation.

Cell Surface Antigen Staining (Optional)

Cell surface antigen staining was performed according to experimental requirements.

Cell Fix and Permeabilize

Cell fix and permeabilize process could be operate with steps 3.1 and 3.2 at the same time.

2.1 Prepare 1×EdU Permeabilization/ Wash Buffer (1×EPWB): add 9 ml 1×PBS containing 1%BSA to 1 ml 10×EPWB.

2.2 Suspend the cells with 1 ml 1×PBS. Centrifuge at 200×g for 5 min. Discard the supernatant to collect cells. Repeat once.

2.3 Add 100 µl EdU Fixation Buffer (EFB). Fix at room temperature for 15 min.

2.4 Centrifuge 200×g for 5 min. Discard the supernatant.

2.5 Suspend the cells with 1 ml 1×PBS. Centrifuge at 200×g for 5 min. Discard the supernatant to collect cells.

2.6 Add 100 µl 1×EPWB. Incubate at room temperature for 15 min.

EdU Detection

3.1 Prepare EdU Buffer Additive (EBA) Solution: add 1 ml deionized water to each tube of EBA, vortex and mix until completely dissolved. It is recommended to aliquot the solution before the first use to avoid oxidative degradation caused by repeated use. The solution will precipitate when placed at 2-8°C, which is normal and can be completely dissolved by vortex. If the solution turns brownish-yellow, this indicates that degradation has occurred and needs to be replaced.

3.2 Prepare 1×EBA: add 100 µl 10×EBA to 900 µl ultra-pure water.

3.3 Prepare Staining Solution: Add 1×PBS, Catalyst Solution (CS), 488 Fluorophore-FC and 1×EBA solution in EP tube in sequence, as shown in the following table. Mix well by pipetting. Place on ice. It is recommended to use within 30 min.

1×PBS	440 µl
CS	5 µl
488 Fluorophore-FC	5 µl
1×EBA	50 µl
Total	500 µl

3.4 Add 500 µl staining solution to the cell suspension prepared in Step 2.6. Suspend the cells. Incubate at room temperature for 30 min in the dark.

3.5 Centrifuge at 200×g for 5 min. Discard the staining solution.

3.6 Suspend the cells with 1 ml 1×EPWB. Centrifuge at 200×g for 5 min. Discard the supernatant and collect the cells. Repeat twice.

3.7 Suspend the cells with 500 µl 1×EPWB.

Other Staining(Optional)

Intracellular antigen staining is performed according to experimental requirements.

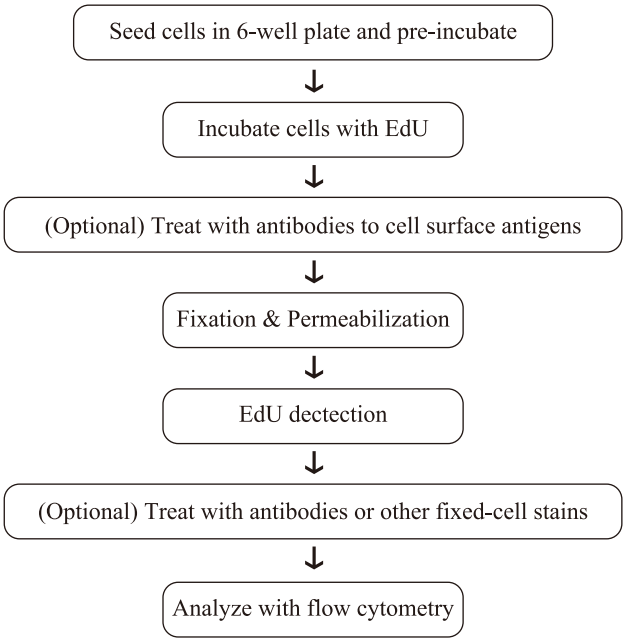
Flow Detection and Analysis

Analyze with flow cytometry, select the appropriate voltage, and adjust the light compensation if performing multicolor analysis. The maximum excitation wavelength and emission wavelength of 488 Fluorophore are 494 nm and 520 nm respectively.

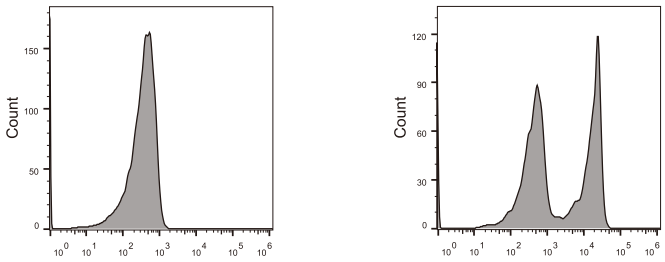
Notes

- Before the experiment, all components should be balanced to room temperature. Ensure that all components are fully dissolved and mixed, and then used after point centrifugation.
- The number of cells used in different cell culture plates/dishes varies, and the ratio can be adjusted according to the operation procedure.
- After cell fixation, antigen staining should be diluted and cleaned by 1×EPWB.
- EBA solution can be stored at 2-8°C for 3 months, and -20°C is recommended for long-term storage.

Operation Flow Chart



TransDetect® EdU Flow Cytometry Kit-488 Fluorophore for detection of A549 cell proliferation



Negative-488 Fluorophore EdU-488 Fluorophore

For research use only, not for clinical diagnosis.

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