TransDetect® EdU Imaging Kit-488 Fluorophore

Please read the datasheet carefully prior to use.

Cat. No. FU101

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. Fluorescence microscopy 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-I	100 µl
EdU Reaction Buffer (ERB)	75 ml
Catalyst Solution (CS)	300 µl
EdU Buffer Additive (EBA)	4×200 mg
Hoechst	60 µl

Procedures

Self-prepared

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

Formaldehyde fixative solution (1×PBS containing 4% paraformaldehyde)

Cell permeabilization solution (1×PBS with 0.1% Triton X-100)

18 mm × 18 mm coverslips

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 with 18 mm×18 mm coverslip 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.

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

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 Remove cell medium and wash cells 1-2 times with 1 ml 1×PBS.

2.2 Add 1 ml formaldehyde fixative solution to per well and fix at room temperature for 15 minutes.

2.3 Remove the fixation solution and wash per well cells 3 times with 1 ml 1×PBS.

2.4 Remove PBS and add 1 ml of cell permeabilization solution at room temperature for 10 minutes.

2.5 Remove permeabilization solution, and wash per well 3 times with 1 ml 1×PBS. The treated cells can be stored in PBS temporarily.

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 Dyeing Solution: add EdU Reaction Buffer (ERB), Catalyst Solution (CS), 488 Fluorophore-I and EBA solution in EP tube in sequence, as shown in the following table, and mix upside-down. After preparation, place it on ice and use it in 30 minutes.

ERB	1132 μl
CS	6 μl
488 Fluorophore-I	2 μl
EBA	60 μl
Total	1.2 ml

3.3 Remove PBS in Step 2.5, add 1.2 ml of staining solution, and incubate at room temperature for 30 minutes in the dark.

3.4 Remove the staining solution and wash the cells 3 times with 1 ml 1×PBS. The treated cells can be stored in PBS temporarily.

Other Staining(Optional)

Intracellular antigen staining is performed according to experimental requirements.

DNA Staining

4.1 Prepare Hoechst Reaction Solution: dilute Hoechst with 1×PBS in a ratio of 1000:1.

4.2 Add 1.2 ml Hoechst Reaction Solution to each well and incubate at room temperature for 15 minutes in the dark.

4.3 Remove the reaction solution, and wash the cells 3 times with 1 ml 1×PBS. The treated cells can be stored in PBS temporarily.

Image Acquisition and Analysis

The maximum excitation wavelength and emission wavelength of 488 Fluorophore are 494 nm and 520 nm respectively for fluorescence microscopy.

The dosage and preparation of EdU medium and staining working solution in different cell culture plates are shown in the table below:

Table 1 Dosage of EdU medium and staining solution in different cell culture plates

	96-well	48-well	24-well	12-well	6-well
Single Well Area	0.3 cm^2	1 cm^2	2 cm^2	4 cm^2	10 cm^2
EdU Culture Medium	100 µl	250 µl	500 µl	1 ml	2 ml
Staining Solution	100 µl	200 µl	300 µl	600 µl	1.2 ml

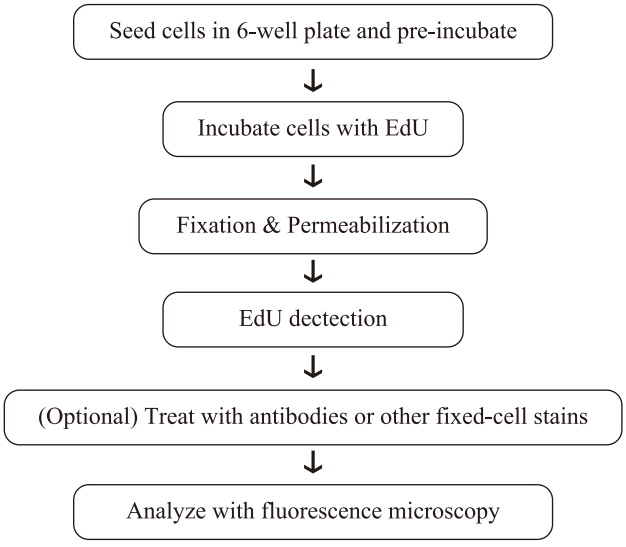
Table 2 Reference for preparation of staining solution with different dosage

	100 µl	200 µl	300 µl	600 µl	1.2 ml
ERB	94 µl	189 µl	283 µl	566 µl	1132 µl
CS	0.5 µl	1 µl	1.5 µl	3 µl	6 µl
488 Fluorophore-I	0.2 µl	0.4 µl	0.5 µl	1 µl	2 µl
EBA	5 µl	10 µl	15 µl	30 µl	60 µl

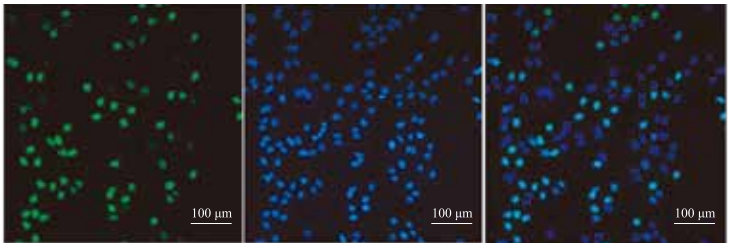
Notes

- 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. It can be adjusted proportionally according to the operation steps.
- 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 Imaging Kit-488 Fluorophore for detection of A549 cell proliferation



488 Fluorophore Hoechst Merge

For research use only, not for clinical diagnosis.

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