BrdU Cell Proliferation Colorimetric Assay (200 tests) (TBS2086)

Description

BrdU Cell Proliferation Colorimetric Assay Kit is designed as a precise, fast and simple colorimetic alternative to quantitate cell proliferation based on the measurement of BrdU incorporation during DNA synthesis in proliferating cells.

5-bromo-2-deoxyuridine (BrdU) is a pyrimidine analog. It gets incorporated into the newly synthesized DNA of proliferating cells in place of thymidine. Tribioscience's BrdU Cell Proliferation Colorimetric Assay Kit detects incorporated BrdU using an anti-BrdU antibody conjugate with Peroxidase (POD) and its substrate. The BrdU antibody POD conjugate allows direct detection of incorporated BrdU without the need for an additional secondary detection antibody. The extent of color development is proportional to the quantity of BrdU incorporated into the cells and can be used directly as an indicator of cell proliferation. Compared to other cell proliferation assays, this kit detects only the proliferating cells and not the seeded cells. This highly sensitive, non-radioactive kit detects as less as 50-100 proliferating cells.

Kit Components and Storage for 200 tests

Part #	Name	Unit Size
A	1000x Brdu Labeling Reagent	30 µL
B	100x Anti-BrdU-POD	300 µL
C	Antibody Diluent	20 mL
D	10x Washing Buffer	25 mL
E	FixDenat, Ready-to-use	45 mL
F	TMB Substrate	25 mL
Storage at –20°C.		
Shelf-life: 6 month after receipt.		

Applications

- Detection and quantification of cell proliferation induced by growth factors, cytokines, mitogens, and nutrients.
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Determination of the inhibitory or stimulatory effects of various compounds on cell proliferation.

Reagents and Equipment Not Provided

- 96-well plate with flat bottom (tissue cell culture treated)
- Multi-well microplate reader
- Stop Solution: 1M H_2SO_4.

Sample Types

- Adherent and Suspension cells.

Preparation Instructions

10x BrdU labeling stock solution: Dilute BrdU labeling reagent (Part A) 1:100 with sterile culture medium resulting a 10x BrdU labeling stock solution. (*Note: The labeling stock solution is stable for several weeks store protected from Light at 2-8°C*).

1x Anti-BrdU-POD working solution: Dilute anti-BrdU-POD Stock Solution (Part B) 1:100 with Antibody Diluent (Part C). Note: Prepare shortly before use! Do not store!

1x Washing Buffer: Dilute Washing Buffer concentrate (Part D) 1:10 with double dist. water. For one 96-well microplate (MP), dilute 10 mL Washing Buffer concentrate (Part D) with 90 mL double dist. water.

BrdU Cell Proliferation Assay Protocol

1. **Cell Culture:** cells are cultured with the respective test compound in a 96-well plate in a final volume of 100 µL/well at 37°C for required time period depending upon the cell type. Treat cells with desired test compound(s) for 1-72 hrs.
2. **BrdU incorporation:** Add 10 µL of 10X BrdU stock solution into desired wells, incubate plate at 37°C for 2-24 hrs.
3. **Fix and Denature:** Remove labeling medium from cells, add 200 µL of FixDenat Solution (Part E) into each well. Incubate at room temperature for 30 min.
4. **BrdU Detection:** Remove FixDenat solution carefully, add 100 µL of 1X Anti-BrdU-POD working solution into each well. Incubate with gentle shaking at room temperature for 90 min (*Note: the incubation time can be varied between 30-120 min, depending on individual requirements*). Remove antibody conjugate by flicking off and rinse wells with 200-300 µL of 1X Wash Buffer for 3 times.
5. **Measurement:** After washing, add 100 µL Substrate (Part F) into each well and incubate at room temperature until color development for 5-30 min:

5A: Without stop solution: Measure the absorbance of the samples in a microplate reader at370 nm (reference wavelength: 492nm).

5B: With Stop Solution: To stop the color development, add 25µL Stop Solution (1M H_2SO_4, not provided) to each well, then measure absorbance at 450 nm (reference wavelength: 690nm).

Notes: a. For suspension cells, centrifuge plate at 300 x g for 10 min. and remove medium carefully before adding FixDenat; b. Incubation time after addition of Substrate must be optimized to avoid over development of color.

Related Product:

Resazurin Cell Viability Kit (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)
Alkaline Phosphatase Staining kit I-Red (TBS2080)
Alkaline Phosphatase Staining kit II-Blue (TBS2085)
Cytochrome C Oxidate Assay (TBS2115)
Cytochrome c Reductase Activity Assay (TBS2116)
Non-esterified Fatty Acid Assy(TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)

Precautions and Disclaimer

This kit is for R&D use only, not for drug, household, or other uses.