

BrdU Cell Proliferation Assay Kit

6/14

(Catalog # K306-200, -1000; 200, 1000 assays; Store at -20°C)

I. Introduction:

5-bromo-2-deoxyuridine (BrdU) is a pyrimidine analog. It gets incorporated into the newly synthesized DNA of proliferating cells in place of thymidine. Biovision's BrdU Cell Proliferation Assay Kit detects incorporated BrdU using a mouse anti-BrdU antibody. An anti-mouse HRP-linked secondary antibody is used to detect the anti-BrdU antibody bound to BrdU, which is followed by addition of TMB (a HRP substrate). 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.

II. 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.

III. Sample Type:

- Adherent and Suspension cells

IV. Kit Contents:

Components	K306-200	K306-1000	Cap Code	Part Number
BrdU (1000X)	20 µl	100 µl	Amber	K306-xxx(x)-1
Fixing/Denaturing Solution	20 ml	100 ml	WM	K306-xxx(x)-2
BrdU Detection Antibody (300X)	70 µl	350 µl	Green	K306-xxx(x)-3
Anti-mouse HRP-linked Antibody (2000X)	10 µl	50 µl	Orange	K306-xxx(x)-4
Antibody Diluent	50 ml	250 ml	NM	K306-xxx(x)-5
Wash Buffer (10X)	40 ml	200 ml	NM-Blue	K306-xxx(x)-6
TMB Substrate	20 ml	100 ml	Brown	K306-xxx(x)-7
Stop Solution	20 ml	100 ml	NM-Brown	K306-xxx(x)-8

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom (tissue cell culture treated)
- Multi-well microplate reader

VI. Storage Conditions & Reagent Preparation:

Store kit at -20°C protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the experiment. Prepare following reagents as needed just before use. We don't recommend storing the diluted solutions.

- **BrdU:** Prepare 10X BrdU solution by diluting BrdU 1:100 with the cell culture medium.
- **BrdU Detection Antibody:** Prepare 1X solution by diluting BrdU Detection Antibody 1:300 with Antibody Diluent.
- **Anti-mouse HRP-linked Antibody:** Prepare 1X solution by diluting Anti-mouse HRP-linked antibody 1:2000 with Antibody Diluent.
- **Wash Buffer:** Prepare 1X solution by diluting with dH₂O.

VII. BrdU Cell Proliferation Assay Protocol:

1. **Cell Culture:** Plate cells in a 96-well plate and incubate for required time period depending upon the cell type. Treat cells with desired test compound(s) for 1-72 hrs.
2. **BrdU incorporation:** Add 10X BrdU solution into desired wells to a final concentration of 1X. Incubate plate at 37°C for 1-4 hrs.
Note: Seed cells at a density of 2500-10,000 cells/well depending on the cell growth rate. Incubation time needs to be optimized for each cell line.
3. **BrdU Detection:** Remove medium from cells & add 100 µl of Fixing/Denaturing Solution into each well. Incubate at room temperature for 30 min. Remove solution carefully & add 100 µl of 1X BrdU Detection Antibody solution into each well. Incubate at room temperature for 1 hr with gentle shaking. Remove solution & wash wells with 300 µl 1X Wash Buffer (2 times). After washing, add 100 µl of 1X Anti-mouse HRP-linked Antibody Solution into each well and incubate the plate at room temperature for 1 hr. Remove solution and wash wells with 300 µl of 1X Wash Buffer (3 times).
4. **Measurement:** Add 100 µl TMB Substrate into each well & measure the absorbance at 650 nm for 5-30 min. at room temperature to monitor the color development. To stop the color development, add 100 µl Stop Solution into each well & measure absorbance at 450 nm.

Notes:

- a. For suspension cells, centrifuge plate at 300 x g for 10 min. and remove medium carefully before adding Fixing/Denaturing Solution.
- b. Incubation time after addition of TMB substrate must be optimized to avoid over development of color. Recommended absorbance is ~0.8-1. After addition of stop solution, read plate immediately.
- c. The value from OD 450 nm will be roughly double of that from OD 650 nm.

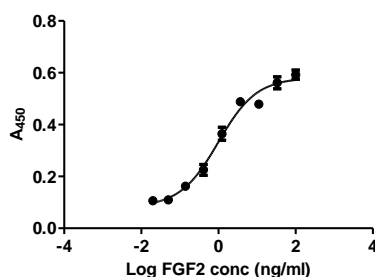


Figure: Treatment of Balb/3T3 cells with h-FGF2 increases cell proliferation as detected by BrdU Cell Proliferation Assay. The cells were seeded at 5000 cells/well in a 96-well plate and incubated overnight at 37°C followed by an overnight incubation in serum-free media at 37°C. h-FGF2 was then added into the plate at different concentrations and cells were incubated for 24 hrs at 37°C. 1X BrdU solution was added into each well and the cells were further incubated for 4 hrs.

VII. Related Products:

Quick Cell Proliferation Colorimetric Assay Kit (K301)
Ready-to-use Cell Proliferation Reagent, WST-1 (K304)
MTS Cell Proliferation Colorimetric Assay Kit (K300)
WST-1 (2198)
Anti-BrdU antibody (Clone B1G5, 3618)
ApoBrdU Red DNA Fragmentation Kit (K404)

Quick Cell Proliferation Colorimetric Assay Kit plus (K302)
VisionBlue™ Quick Cell Viability Fluorometric Assay Kit (K303)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
ApoBrdU DNA Fragmentation Assay Kit (K401)
ApoBrdU-IHC DNA Fragmentation Assay Kit (K403)

FOR RESEARCH USE ONLY! Not to be used on humans.