BrdU Cell Proliferation Microplate Assay Kit User Manual

Catalog # CAK2010

(Version 1.1A)

Detection and quantification of cell proliferation in adherent and suspension cells samples.

For research use only. Not for diagnostic or therapeutic procedures.

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.

BrdU Cell Proliferation Assay Kit detects incorporated BrdU using a rabbit anti-BrdU antibody. A Goat Anti-Rabbit IgG (H&L) - HRP secondary antibody is used to detect the anti-BrdU antibody bound to BrdU, which is followed by addition of TMB. 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.

II. KIT COMPONENTS

Component	Volume	Storage
BrdU (1000X)	10 µl x 1	4 °C
Fixing Solution	10 ml x 1	4 °C
Denaturing Solution	10 ml x 1	4 °C
BrdU Antibody (1000X)	20 µl x 1	-20 °C
Goat Anti-Rabbit IgG (H&L) - HRP (2000X)	10 µl x 1	-20 °C
Antibody Diluent	30 ml x 1	4 °C
Wash Buffer (20X)	30 ml x 1	4 °C
TMB Substrate	12 ml x 1	4 °C
Stop Solution	12 ml x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. 96-well clear plate with flat bottom (tissue cell culture treated)

IV. SAMPLE PREPARATION

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.

V. REAGENT PREPARATION

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 Antibody (1000X): Prepare 1X solution by diluting BrdU Detection Antibody 1:1000 with Antibody Diluent.

Goat Anti-Rabbit IgG (H&L) - HRP (2000X): Prepare 1X solution by diluting the Goat Anti-Rabbit IgG (H&L) - HRP (2000X) 1:2000 with Antibody Diluent.

Wash Buffer (20X): Prepare 1X solution by diluting with dH2O.

VI. ASSAY PROCEDURE

1. BrdU incorporation: Add 10X BrdU solution into desired wells to a final concentration of 1X. Incubate plate at 37°C for 1-4 hrs in dark.

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.

2. Fixing the cells: Remove medium from cells. Add 100 µl of Fixing Solution into each well. Incubate at room temperature for 30 min. Remove solution carefully. Add 100 µl of Denaturing Solution into each well. Incubate at room temperature for 30 min. Remove solution carefully.

3. BrdU Detection: Add 100 µl of 1X BrdU Antibody solution into each well. Incubate at room temperature for 1 hr with gentle shaking. Remove solution and wash wells with 300 µl 1X Wash Buffer (2 times). After washing, add 100 µl of 1X Goat Anti-Rabbit IgG (H&L) - HRP 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 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 and 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.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES