Product Manual

CytoSelect™ BrdU Cell Proliferation ELISA Kit

Catalog Number

CBA- 251 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The investigation of cell cycle and DNA synthesis has been essential to many fields of science. Traditionally a radiolabled thymidine has been used to track new DNA synthesis and cellular proliferation. Although quite sensitive, use of radiolabled thymidine has the limitation of having to regulate, handle and dispose of radioisotopes and often requires expensive detection equipment.

More recently, the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) has been used in place of radiolabled thymidine and is incorporated into newly synthesized DNA strands of actively proliferating cells. Following fixation and partial denaturation of cellular DNA, BrdU can be detected immunochemically which allows for the analysis of live cell new DNA synthesis.

Assay Principle

The CytoSelect™ BrdU Cell Proliferation ELISA Kit detects BrdU incorporated into cellular DNA during cell proliferation using an anti-BrdU antibody. When cells are incubated in media containing BrdU, the pyrimidine analog is incorporated in place of thymidine into the newly synthesized DNA of proliferating cells (Figure 1). Once the labeling media is removed, the cells are fixed and the DNA is denatured in one step with a fix/denature solution (denaturation of the DNA is necessary to improve the accessibility of the incorporated BrdU for detection). Then an anti-BrdU mouse monoclonal antibody is added followed by an HRP conjugated secondary antibody to detect the incorporated BrdU. The magnitude of the absorbance for the developed color is proportional to the quantity of BrdU incorporated into cells and can be directly correlated to cell proliferation.

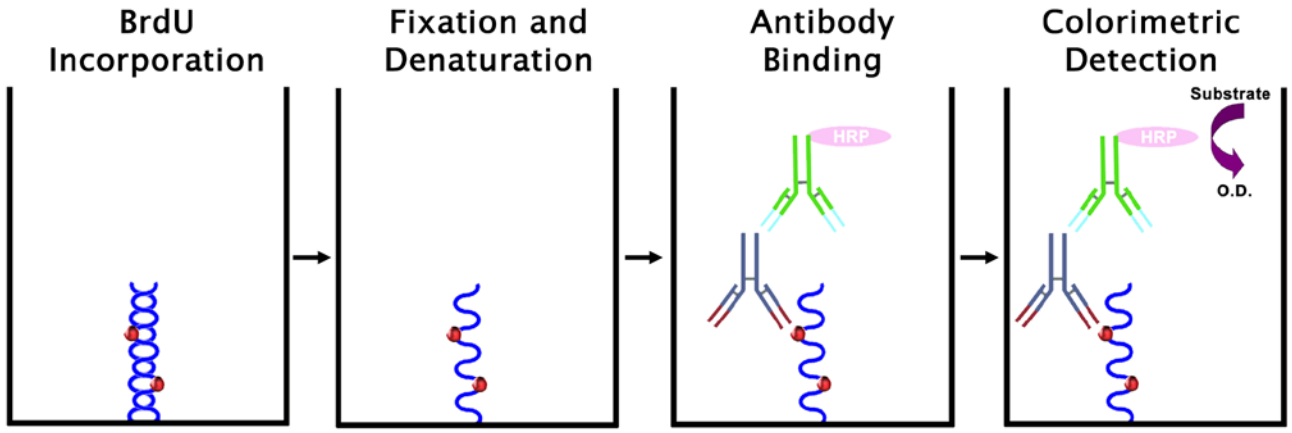

Figure 1: Schematic of the CytoSelect™ BrdU Proliferation ELISA

Related Products

1. CBA-240 CytoSelect™ Cell Viability and Cytotoxicity Assay
2. CBA-081 CytoSelect™ 96-Well Anoikis Assay
3. CBA-253 CytoSelect™ Cell Proliferation Assay Reagent (Colorimetric)
4. CBA-250 CytoSelect™ Cell Proliferation Assay Reagent (Fluorometric)

Kit Components

1. 1000X BrdU Solution (Part No. 125101): One 30 µL vial of 10 mM BrdU.
2. Anti-BrdU Monoclonal Antibody (Part No. 125102): One 10 µL vial of mouse anti-BrdU antibody.
3. Fix/Denature Solution (Part No. 125103): One 20 mL bottle.
4. Antibody Diluent (Part No. 125104): One 50 mL bottle.
5. Secondary Antibody, HRP Conjugate (Part No. 230003): One 20 µL vial.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Materials Not Supplied

1. Mammalian Cells
2. Cell growth media
3. PBS
4. Deionized water
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
7. 96 well cell culture plate
8. Multichannel micropipette reservoir
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store the 1000X BrdU Solution and Anti-BrdU Monoclonal Antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-BrdU Monoclonal Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-BrdU Monoclonal Antibody 1:1000 with Antibody Diluent. Immediately before use dilute the Secondary Antibody, HRP Conjugate 1:1000 with Antibody Diluent. Do not store diluted solutions.
- 10X BrdU Solution: Immediately before use, dilute 1000X stock of BrdU 1:100 with cell growth media.

Assay Protocol

1. Prepare a cell suspension containing 0.1-1.0 x 10^6 cells/ml in medium.

2. Add 100 µL per well to a 96-well cell culture plate and incubate overnight at 37°C and 5% CO_2 in a humidified incubator.
3. Add compound to be tested and include wells without compound (or with vehicle) as a negative control. Culture the cells for 24-96 hours at 37°C and 5% CO_2 in a humidified incubator.
4. Add 10 µL of 10X BrdU Solution (see Preparation of Reagents Section) to wells and incubate at 37°C and 5% CO_2 in a humidified incubator for 1-6 hours.

   Note: optimal time of incubation with BrdU will vary with cell type.
5. Carefully and slowly aspirate wells by pipette and add 100 µL PBS; repeat this wash step 2 more times.
6. After the final aspiration, add 100 µL Fix/Denature Solution and incubate 30 minutes at 37°C.
7. Wash wells 3 times 100 µL per well with PBS as described in step 5.
8. Add 100 µL Antibody Diluent and incubate 1 hour at room temperature.
9. Wash wells 3 times with 100 µL PBS.
10. Add 100 µL of diluted Anti-BrdU Antibody (see Preparation of Reagents section) to each tested well. Incubate at room temperature for 1 hour on an orbital shaker.
11. Wash wells 3 times with 250 µL 1X Wash Buffer per well as described above for PBS washes.
12. Add 100 µL of the diluted Secondary Antibody HRP Conjugate (see Preparation of Reagents section) to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.
13. Wash the strip wells 3 times according to step 11. Proceed immediately to the next step.
14. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
15. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
16. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical CytoSelect™ BrdU Cell Proliferation ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

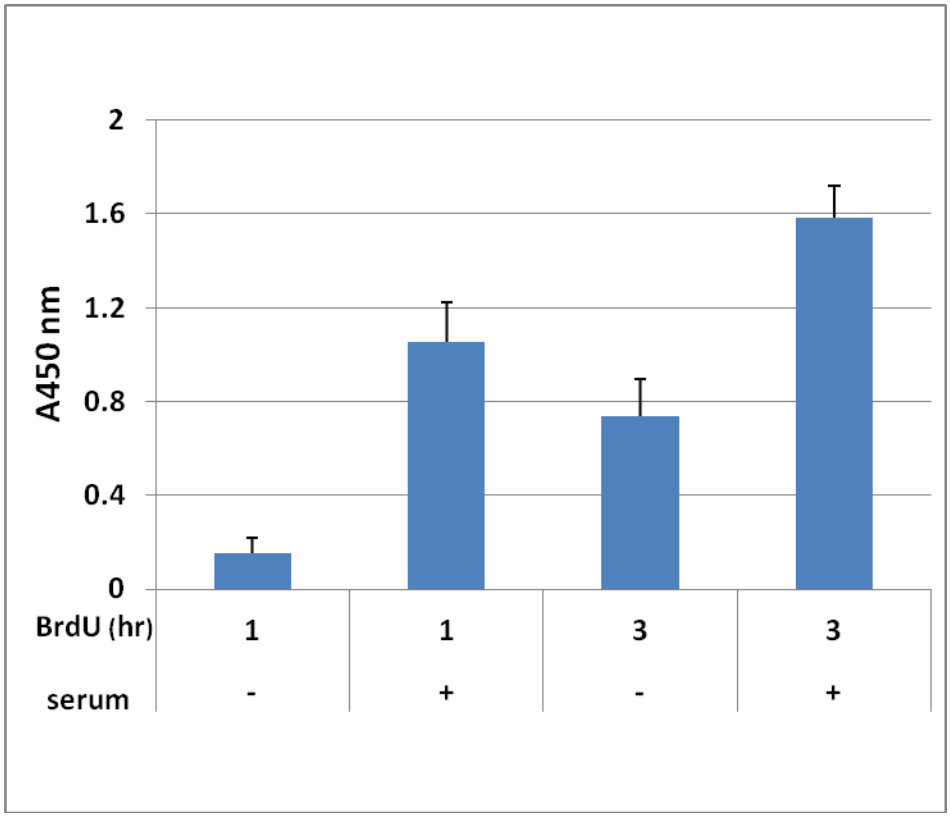

Figure 2: Serum Stimulation of Proliferation in HEK 293 Cells. HEK 293 Cells were plated overnight at 37ºC. Cells were then incubated in the presence or absence of 10% FBS for 24 hours, treated with 10 µM BrdU for 1 or 3 hours, and processed for BrdU incorporation according to the assay protocol.

References

1. Shoghi KI, Xu J, Su Y, He J, Rowland D, Yan Y, Garbow JR, Tu Z, Jones LA, Higashikubo R, Wheeler KT, Lubet RA, Mach RH, You M.. (2013) *PLoS One.* **8**:e74188
2. Gong F, Wang G, Ye J, Li T, Bai H, Wang W (2013) *Oncol Rep.* **30**:2976-2982
3. Piastowska-Ciesielska AW, Kozłowski M, Wagner W, Domińska K, Ochędalski T. (2013) *Arch Med Sci.* **30**:739-744.
4. Liang XH, Li LL, Wu GG, Xie YC, Zhang GX, Chen W, Yang HF, Liu QL, Li WH, He WG, Huang YN, Zeng XC. (2013) *BMC Cancer* **13**:412
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Recent Product Citations

1. Maglione, M. et al. (2017). In vivo evaluation of chitosan-glycerol gel scaffolds seeded with stem cells for full-thickness mandibular bone regeneration. *J. Oral Sci.* **59(2)**:225-232.
2. Kim, B. S. et al. (2016). D-dopachrome tautomerase in adipose tissue inflammation and wound repair. *J Cell Mol Med*. doi:10.1111/jcmm.12936.
3. Kreiseder, B. et al. (2015). Alpha-catulin contributes to drug-resistance of melanoma by activating NF-κB and AP-1. *PLoS One*. **10**:e0119402-e0119402.
4. Hatzis, C. et al. (2014). Enhancing reproducibility in cancer drug screening: how do we move forward? *Cancer Res*. **74**:4016-4023.

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