BrdU Cell Proliferation Assay Kit

#6813 Store at –20°C

✔ 1 Kit
(500 assays (96 well format))

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Description: The BrdU Cell Proliferation Assay Kit detects 5-bromo-2'-deoxyuridine (BrdU) incorporated into cellular DNA during cell proliferation using an anti-BrdU antibody. When cells are cultured with labeling medium that contains BrdU, this pyrimidine analog is incorporated in place of thymidine into the newly synthesized DNA of proliferating cells. After removing labeling medium, cells are fixed and the DNA is denatured with our fixing/denaturing solution. Then a BrdU mouse mAb is added to detect the incorporated BrdU (The denaturing of DNA is necessary to improve the accessibility of the incorporated BrdU to the detection antibody). Anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate TMB is added to develop color. The magnitude of the absorbance for the developed color is proportional to the quantity of BrdU incorporated into cells, which is a direct indication of cell proliferation.

Specificity/Sensitivity: BrdU Cell Proliferation Assay kit detects BrdU incorporation into cellular DNA during cell proliferation. The BrdU-labeled DNA has to be denatured to be detected by the BrdU Mouse mAb used in this kit. This BrdU Mouse mAb does not cross react with endogenous DNA. Depending on the cell type and the incubation time applied in the assay, $0.2\text{-}2x10^4$ cells/well are sufficient for most experimental setups. For the best result, a cell number titration (Figure 1) is recommended.

Background: Halogenated nucleotides such as the pyrimidine analog bromodeoxyuridine (BrdU) are useful for labeling nascent DNA in living cells and tissues. BrdU becomes incorporated into replicating DNA in place of thymidine and subsequent immunodetection of BrdU using specific monoclonal antibodies allows labeling of cells in S phase of the cell cycle. After pulse-labeling cells or tissues with bromodeoxyuridine, BrdU (Bu20a) Mouse mAb can be used to detect BrdU incorporated into single stranded DNA. Please see our detailed protocol for information regarding the labeling procedure as well as denaturation of double stranded DNA for various immunodetection applications (1-4).

Background References:

(1) Darzynkiewicz, Z. and Juan, G. (2001) *Curr Protoc Cytom* Chapter 7, Unit 7.7.

(2) Leif, R.C. et al. (2004) *Cytometry A* 58, 45-52.

(3) Staszkiewicz, J. et al. (2009) *Biochem Biophys Res Commun* 378, 539-44.

(4) Rothaeusler, K. and Baumgarth, N. (2007) *Curr Protoc Cytom* Chapter 7, Unit7.31.

Products Included	Volume	Solution Color
BrdU	0.15 ml	colorless
Fixing/Denaturing Solution	2 x 25 ml	colorless
BrdU Detection Antibody	0.5 ml	green
Anti-mouse IgG, HRP-linked Antibody	0.5 ml	red
Detection Antibody Diluent	50 ml	green
HRP-linked Antibody Diluent	50 ml	red
TMB Substrate	50 ml	colorless
STOP Solution	50 ml	colorless
20X Wash Buffer	50 ml	colorless

Note: This kit contains mixed storage components. Please store this entire kit at -20° C for long term storage. Upon first use, please allow components to thaw and then store each component as indicated on individual component labels.

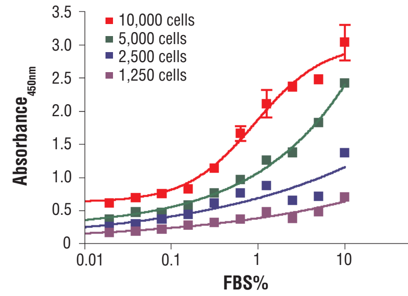

Figure 1: C2C12 cells were seeded at varying density (in serum free medium) as shown in the figure in a 96-well plate and incubated overnight. Serum was added to the plate at various concentration as shown in the figure and cells were incubated for 24 hours. Finally, 10 µM BrdU was added to the plate and cells were incubated with BrdU containing medium for 4 hours.

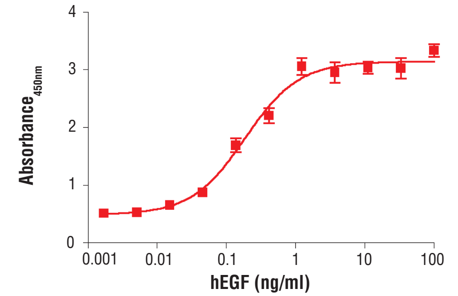

Figure 2: Treatment of MCF 10A cells with Human Epidermal Growth Factor (hEGF) #8916 increases cell proliferation as detected by BrdU Cell Proliferation Assay Kit #6813. MCF 10A cells were seeded at 1x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were then starved in serum free medium overnight. hEGF was added to the plate and cells were incubated for 24 hours. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hours.

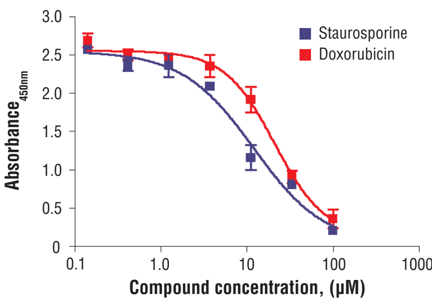

Figure 3. Jurkat cells were seeded at 4×10^4 cells/well in a 96-well plate and incubated overnight. Cells were then treated with various concentrations of doxorubicin (red) or staurosporine (blue) for 2 hours. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hours.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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BrdU Cell Proliferation Protocol

A Reagent Preparation

1. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each BrdU ELISA Kit) in Milli-Q or equivalently purified water.
2. Prepare 1X detection antibody solution by diluting the 100X BrdU detection antibody in the Antibody Dilution solution (Green).
3. Prepare 1X HRP-conjugated secondary antibody solution by diluting the 100X stock solution in HRP-conjugate Dilution solution (Red).

B BrdU Incorporation:

1. Plate cells in 96-well plate and incubate with respective test substance. Typical seed cell number is 2500-100000 cells/well depending on cell growth rate. Typical incubation time is 1-72 hours.
2. Prepare BrdU solution by diluting the BrdU 1000X stock in cell culture medium at 1:100 diluiton. Please note that the final BrdU concentration should be 10 µM. (Example: For 100 µl medium in the plate, add 10 µl of BrdU solution per well.) The kit contains enough BrdU for 500 assays (based on 200 µl/well medium volume).
3. Place cells in incubator. Typical incubation time is 1-24 hours.
4. Remove medium. For suspension cells, centrifuge the plate at 300 g for 10 minutes, then remove medium.

C BrdU Assay:

1. Add 100 µl /well of the fixing/denaturing solution, keep the plate at room temperature for 30 minutes. Remove solution.
2. Add 100 µl /well premade 1X Detection antibody solution, keep plate at room temperature for 1 hour. Remove solution and wash plate 3 times with 1X wash buffer.
3. Add 100 µl/well prepared 1X HRP-conjugate solution and keep plate at room temperature for 30 minutes. Remove the solution and wash plate 3 times with 1X wash buffer.
4. Add 100 µl TMB substrate.
5. Incubate for 30 minutes at room temperature.

Note: Watch the color change as it may be necessary to stop the reaction prior to the standard development time of 30 minutes.

6. Add 100 µl STOP solution.
7. Read absorbance at 450 nm (For optimal readings, please read the plate within 30 minutes after adding STOP solution).

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