Store at –20°C

#5492

BrdU Cell Proliferation Chemiluminescent Assay Kit

1 Kit
(500 assays (96 well format))

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

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. Denaturing of DNA is necessary to improve the accessibility of the incorporated BrdU to the detection antibody. A BrdU mouse mAb is then added to detect the incorporated BrdU. Anti-mouse IgG, HRP-linked Antibody is used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of BrdU incorporated into cells, which is a direct indication of cell proliferation.

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 and denaturation of double stranded DNA for various immunodetection applications (1-4).

Specificity/Sensitivity: BrdU Cell Proliferation Chemiluminescent Assay Kit detects BrdU incorporation into cellular DNA during cell proliferation. The BrdU-labeled DNA must 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, 0.2-2x10^4 cells/well are sufficient for most experimental setups. For best results, a cell number titration (Figure 1) is recommended.

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.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
BrdU (1000X)	75953	0.15 ml		-20°C
Fixing/Denaturing Solution (1X)	32375	2 x 25 ml		RT
BrdU Detection Antibody (100X)	94079	0.5 ml	Green	-20°C
Anti-mouse IgG, HRP-linked Antibody (100X)	34709	0.5 ml	Red	-20°C
Detection Antibody Diluent	13339	50 ml	Green	4°C
HRP-linked Antibody Diluent	13515	50 ml	Red	4°C
Luminol/Enhancer Solution	84850	25 ml		RT
Stable Peroxide Buffer	42552	25 ml		RT
20X Wash Buffer	48969	50 ml		4°C

Note: This kit contains mixed storage components. Please store this entire kit at -20C for long term storage. Upon first use, please allow components to thaw and then store each component as indicated in the chart above and on individual component labels. All components in this kit are stable for at least 24 months when stored at the recommended temperature and left unused.

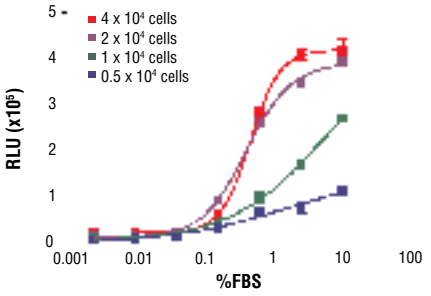

Figure 1. C2C12 cells were seeded at varying density in serum free medium in a 96-well plate and incubated overnight. Serum was added to the plate at various concentrations and cells were incubated for 24 hr. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hr.

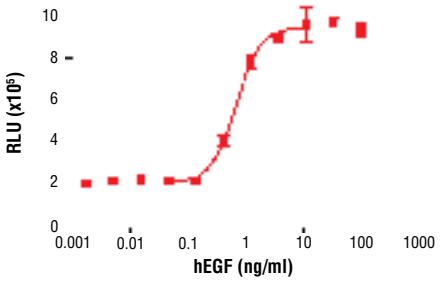

Figure 2. Treatment of MCF 10A cells with Human Epidermal Growth Factor (hEGF) #8916 increases cell proliferation as detected by the BrdU Cell Proliferation Chemiluminescent Assay Kit #5492. MCF 10A cells were seeded at 1x10^4 cells/well in a 96-well plate and incubated overnight. Cells were then starved in serum free medium overnight. hEGF was added to the plate and cells were incubated for 24 hr. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hr.

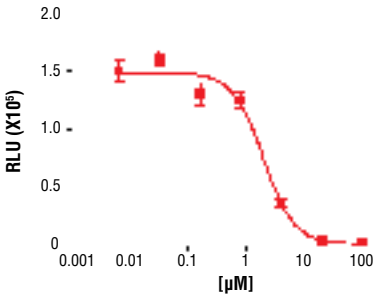

Figure 3. Jurkat cells were seeded at 5x10^4 cells/well in a 96-well plate and incubated overnight. Cells were then treated with various concentrations of doxorubicin for 2 hr. Finally, 10 µM BrdU was added to the plate and cells were incubated for 4 hr.

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.

BrdU Cell Proliferation (Chemiluminescent Assay) Protocol

A Reagent Preparation

1. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each BrdU ELISA Kit) in purified water.
2. Prepare 1X detection antibody solution by diluting BrdU Detection Antibody 1:100 with Detection Antibody Diluent (green).
3. Prepare 1X HRP-conjugated secondary antibody solution by diluting Anti-mouse IgG, HRP-linked Antibody 1:100 with HRP-linked Antibody Diluent (red).
4. Prepare 10X BrdU solution by diluting BrdU 1:100 with cell culture medium.

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 hr.
2. Add prepared 10X BrdU solution to plate wells, for a final 1X concentration. (Example: For 100 µl medium in the plate, add 10 µl of 10X BrdU solution per well.)
3. Place cells in incubator. Typical incubation time is 1-24 hr.
4. Remove medium. For suspension cells, centrifuge the plate at 300 g for 10 min, then remove medium.

C BrdU Assay:

1. Add 100 µl/well of the Fixing/Denaturing Solution, keep the plate at room temperature for 30 min. Remove solution.
2. Add 100 µl/well prepared 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-conjugated secondary antibody solution, keep plate at room temperature for 30 min. Remove the solution and wash plate 3 times with 1X Wash Buffer.
4. Prepare Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
5. Add 100 µl of the Working Solution to each well.
   Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nM within 1-10 min following addition of the substrate.
   Optimal signal intensity is achieved when read within 10 min.