Bromodeoxyuridine (BrdU)
Cell Proliferation Assay Kit Manual

Features

- High Sensitivity - <50 cells/well
- Fix/store and assay later
- 4ºC Storage - long shelf life
- Non-radioactive
- 2.5 hour protocol
- Colorimetric format
- HTS format compatible

Ordering Information

Catalog Number
X1327K

Size
200, 1000 and 5000 Tests

Format
ELISA Kit

Species Reactivity
Ubiquitous

Company Information
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Other ELISA Kits Available from Exalpha Biologicals

BrdU Cell Proliferation ELISA Kit

200 tests (Cat. No. X1327K1)

1000 tests (Cat. No. X1327K2)

5000 tests (Cat. No. X1327K3)

BrdU Immunohistochemistry Kit

50 sections (Cat. No. X1545K)

PIG3 (p53 Inducible Gene-3) ELISA Kit

1 Plate (Cat. No. X1326K1)

2 Plates (Cat. No. X1326K2)

Mouse TNF ELISA Kit

1 Plate (Cat. No. X1851K)

Mouse IL-17 ELISA Kit

1 Plate (Cat. No. X2748K)

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BrdU

Cell Proliferation Assay

Intended Use

The Exalpha Biologicals, Inc. BrdU Cell Proliferation Assay is a non-isotopic immunoassay for the quantitation of bromodeoxyuridine incorporation into newly synthesized DNA of actively proliferating cells.

This assay is for research use only and not for use in diagnostic or therapeutic procedures.

Storage of Kit Components

The Exalpha Biologicals BrdU Cell Proliferation Assay kit components are shipped on blue ice. Upon receipt, store entire kit at 4-8ºC.

Before first use:
Remove the Fixative/Denaturing Solution and place at room temperature for at least 4 hours prior to use. Precipitates that may occur while cold should go back into solution.

Background

A non-isotopic enzyme immunoassay for the quantification of DNA synthesis and cell proliferation.

Evaluation of cell cycle progression is essentialfor investigations in many scientific fields. Measurement of [3H] thymidine incorporation as cells enter S phase has long been the traditional method for the detection of cell proliferation. Subsequent quantification of [3H] thymidine is performed by scintillation counting or autoradiography. This technology is slow, labor intensive and has several limitations including the handling and disposal of radioisotopes and the necessity of expensive equipment. A well-established alternative to [3H] thymidine uptake

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has been demonstrated by numerous investigators (1-8). In these methods bromodeoxyuridine (BrdU), a thymidine analog replaces [3H] thymidine. BrdU is incorporated, into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, BrdU is detected immunochemically allowing the assessment of the population of cells, which are actively synthesizing DNA.

Exalpha Biologicals BrdU Cell Proliferation Assay involves incorporation of BrdU into cells cultured in microtiter plates using the cell layer as the solid phase. The resultant assay is sensitive, rapid, easy to perform and applicable to high sample throughput. In addition to evaluation of cell proliferation, information such as cell number, morphology and analysis of cellular antigens can be obtained from a single culture.

Principle of the Assay

Exalpha Biologicals Proliferation Assay involves incorporation of BrdU into cells cultured in microtiter plates using the cell layer as the solid phase. During the final 2 to 24 hours of culture BrdU is added to wells of the microtiter plate. BrdU will be incorporated into the DNA of dividing cells. To enable antibody binding to the incorporated BrdU cells must be fixed, permeabilized and the DNA denatured. This is all done in one step by treatment with Fixing Solution. Detector anti-BrdU monoclonal antibody is pipetted into the wells and allowed to incubate for one hour, during which time it binds to any incorporated BrdU. Unbound antibody is washed away and horseradish peroxidase-conjugated goat anti-mouse antibody is added, which binds to the Detector Antibody. The horseradish peroxidase catalyzes the conversion of the chromogenic substrate tetra-methylbenzidine (TMB) from a colorless solution to a blue solution (or yellow after the addition of stopping reagent), the intensity of which is proportional to the amount of incorporated BrdU in the cells. The colored reaction product is quantified using a spectrophotometer.

Summary Protocol

1. Cell Plating – no Test Reagent/Drug (skip step 3 below)	• Seed cells at 1-2 x 105 cells/ml, 100 µl/well
2. Cell Plating – withTest Reagent/Drug (see below step 3)	• Seed cells at 0.5-4 x 105 cells/ml, 100 µl/well

3. Addition of Test Reagent(s)/Drug	• Add 100 µl/well, 2X concentration desired
4. Addition of BrdU	Dilute 500X stock BrdU, add 20 µl/well (be sure to include a No BrdU control)
5. Incubate	• 2-24 hours
6. Fix and Denature	
Adherent and Suspension Cells No-Spin Procedure	• Aspirate (or flick) the media from the cell wells • Add 200 µl/well Fixing Solution • Incubate 30 minutes at Room Temp. • Aspirate the Fixing Solution and blot the plates dry.
Suspension Cells Spin Procedure	• Spin the plates for 5 minutes at 1000 rpm. • Aspirate media, add 200 µl/well Fixing Solution. • Incubate for 30 minutes, room temp. • Aspirate the Fixing Solution and blot the plates dry.
7. Wash Step	• Wash X3 with 1X wash buffer and blot dry.
8. Detector Antibody	• Add 100 µl/well of prediluted Detector Antibody.
9. Incubate	• 1 hour at room temp.
10. Wash Step	• Wash X3 with 1X wash buffer and blot dry.
11. Conjugate Addition	• Add 100 µl/well HRP-conjugate
12. Incubate	• Incubate for 30 minutes at room temperature.
13. Wash Step and Final Water Wash	• Wash as above. **Perform a final distilled water wash by flooding the entire plate with distilled water.** Pat dry on absorbent paper towels.
14. Development	• Add 100 µl/well TMB Peroxidase substrate
15. Incubate	• 30 minutes at room temperature in the dark.
16. Stop	• Add 100 µl of acid Stop Solution to every well
17. Read	• Read the at 450/550 nm.

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Materials Provided

The BrdU Cell Proliferation Assay is provided in either a 200, 1000 or 5000 test sizes. Volume listed below at for the 200 test kit.

1. BrdU REAGENT: A 500x solution of BrdU, 15 µl.
2. FIXING SOLUTION: 40 ml
3. Prediluted ANTI-BrdU DETECTING ANTIBODY: 23 ml of prediluted antibody.
4. STOP SOLUTION: 25 ml of 2.5N sulfuric acid.
5. PEROXIDASE GOAT ANTI-MOUSE IgG (2000X) 15 µl.
6. CONJUGATE DILUENT: 25 ml Buffer for dilution of Conjugate.
7. SUBSTRATE: 25 ml, Ready to use tetramethylbenzidine solution.
8. 50X PLATE WASH CONCENTRATE: 50X concentrated solution of buffered Tris and surfactant.

Materials Required But Not Provided

10. 2-20 μl, 20-200 μl and 200-1000 μl precision pipetters with disposable tips
11. Wash bottle or multichannel dispenser for washing
12. 2000 ml graduated cylinder
13. PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4-7H2O, 1.4 mM KH2PO4)
14. Deionized or distilled H2O
15. Spectrophotometer capable of measuring absorbance in 96-well plates using dual wavelength of 450-540 or 450-595 nm or a single read at 450 nm.
16. Tissue culture microtiter plate (96 well culture dish)
17. Sterile reagent troughs
18. Micro syringe filter (0.2 μm)
19. Syringe

Precautions and Recommendations

1. Do not expose reagents to excessive light.
2. Wear disposable gloves and eye protection.
3. Do not use the kit beyond the expiration date.
4. Do not mix reagents from different kits.
5. Do not mouth pipette or ingest any of the reagents.
6. The buffers and reagents used in this kit contain

anti-microbial and anti-fungal reagents. Care should be taken to prevent direct contact with these products.

7. Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
8. Human samples may be contaminated with infectious agents. Do not ingest, expose to open wounds, or breathe aerosols. Dispose of samples properly.

Detailed Protocol

Recommended Controls

Two types of controls are recomended to insure validity of the experiment.

1. Blank: Add only tissue culture media (no cells).
2. Background: Cells are present in the wells but do not add the BrdU Reagent.

3. Cell Plating

Seed cells using a sterile 96-well tissue culture plate, cells are plated at 2 x 105 cells/ml in 100 μl/well of appropriate cell culture media. Some of the wells on the plate should be set aside for several controls. These should include wells that do not receive cells (media alone), and wells which contain cells but will not receive the BrdU reagent (assay background).

4. Addition of Test Reagent

The test reagent can be a cell proliferation enhancer or alternatively, can induce growth inhibition or arrest. The test reagent is diluted to twice the desired final concentration (2X) in the cell media. 100 µl/well is added on top of the cell wells. The test reagent should be titered in the assay to determine optimum concentration for inducing cell proliferation or growth arrest. The length of time for test reagent incubation should also be determined for your system (time course study). BrdU addition (see step 5 below) will occur 2-24 hours prior to the end of the test reagent incubation.

5. Addition of BrdU

BrdU will be incorporated into proliferating cells and should be added at least 2 hours prior to the end of the test reagent incubation period. Better sensitivity and

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signal to noise ratios are obtained when longer BrdU labeling times are used. Dilute the 500X concentrated stock 1:500 by adding 8 µl of BrdU stock to 4 mls of cell media. Pipette 20 µl of the diluted BrdU label to the appropriate wells. Reminder: a series of wells should be set aside that do NOT receive the BrdU label (- BrdU control for determining assay background). Incubate the assay 2-24 hours.

6. Fix and Denature Step and Storage of Fixed Plates

For detection of the BrdU label by the anti-BrdU monoclonal antibody, it is necessary to fix the cells and denature the DNA using a solution provided in this kit (Fixing Solution). There is no need to spin the cells prior to addition of the fixing solution. However, if suspension cells are being used, better precision is obtained if the cell plates are spun in a centrifuge prior to the fix/denature step. Plates may be fixed (see steps 7-8) and stored at 4oC for assay at a later time. Place dried plates in a sealed dry plastic bag, zip-lock type bags or heat sealed plastic bags are suitable for this purpose. Plates are stable for at least one month when properly stored.

7. Adherent and Suspension Cells (No-Spin Procedure)

Aspirate the media from the cell wells (this can be done mechanically or plate can be inverted over appropriate reservoir and blotted on absorbent paper towels). Add 200 µl/well Fixing Solution and incubate at room temperature for 30 minutes. Aspirate the Fixing Solution and blot the plate dry. Note: Fixed plates can be stored for up to 1 month at 4oC if stored in a heat sealed or zip-lock bag. If storing your plates for future use, make sure the plates are blotted well and are very dry (NO Fixing Solution should be left in the wells).

8. Suspension Cells (Spin Fix/Denature Procedure)

Spin the plates in the centrifuge (using appropriate centrifuge microtiter plate holders) for 5 minutes at 1000 rpm. Aspirate the media and add 200 µl/well Fixing Solution. Incubate for 30 minutes at room temperature. Aspirate the Fixing Solution and blot the plates dry. The assay can be run immediately or plates may be stored for future use (see note above).

9. Wash Step

Dilute the 50X Wash Buffer 1:50 by adding 40 mls to 1.96 liters of distilled water. A microtiter plate washer may be used for all wash steps OR a squirt bottle for manual plate washing may also be used. In either case,

the wells should be filled completely with wash buffer. Wash the plate three times with 1X Wash Buffer prior to adding Detector Antibody. Aspirate the wash solution after the final wash and blot dry on paper towels.

10. Preparation and Addition of Detector Antibody

The anti-BrdU monoclonal Detector Antibody is provided as a prediluted solution. Add 100 µl/well and incubate for 1 hour at room temperature.

11. Wash Step

Wash as in Step 9 above.

12. Preparation and Addition of the Peroxidase Goat Anti-Mouse IgG Conjugate

The Peroxidase Goat Anti-Mouse IgG Conjugate is provided as a concentrated stock solution. Dilute the Conjugate 1:2000 by adding 6 µl to 12 mls of Conjugate Diluent provided. Once diluted, this solution should be filtered using a 0.22 µm syringe filter. This lowers the assay background and improves precision. Pipette 100 µl/well and incubate for 30 minutes at room temperature.

13. Wash Step and Final Water Wash

Wash as in Step 9 above. Perform a final water wash by flooding the entire plate with distilled water. Pat dry on absorbent paper towels.

14. Addition of Substrate

Pipette 100 µl/well TMB Peroxidase substrate and incubate for 30 minutes at room temperature in the dark. Positive wells will be visible by a blue color, the intensity of which is proportional to the amount of BrdU incorporation in the proliferating cells.

15. Addition of Stop Solution and Reading of the Plate

Stop the reaction by pipetting 100 µl of acid Stop Solution provided to every well. The color of positive wells will change from blue to bright yellow. Read the plate using a spectrophotometric microtiter plate reader set at a dual wavelength of 450/550 nm (alternatively, 450/540 nm or 450/595 nm may be used or a single read at 450 nm).

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Model Systems

A sensitivity study was performed using the Jurkat (non-adherent) and RH7777 and MCF7 (adherent) cells. Various concentrations of the cells were plated and cultured for 24 hours. The cells were incubated with BrdU Label for 24 hours and incorporated BrdU was detected with the Exalpha BrdU Cell Proliferation Assay. There was a direct relationship between the signal and number of proliferating cells at all cell concentrations (Figure 1). The sensitivity of this assay was determined to be 40 cells/well using the mean signal of zero plus two standard deviations; that is, the smallest number of cells that may be distinguished from zero with 95% confidence. Using a two-hour BrdU labeling, 100 cells/well was also significantly higher than the blank control.

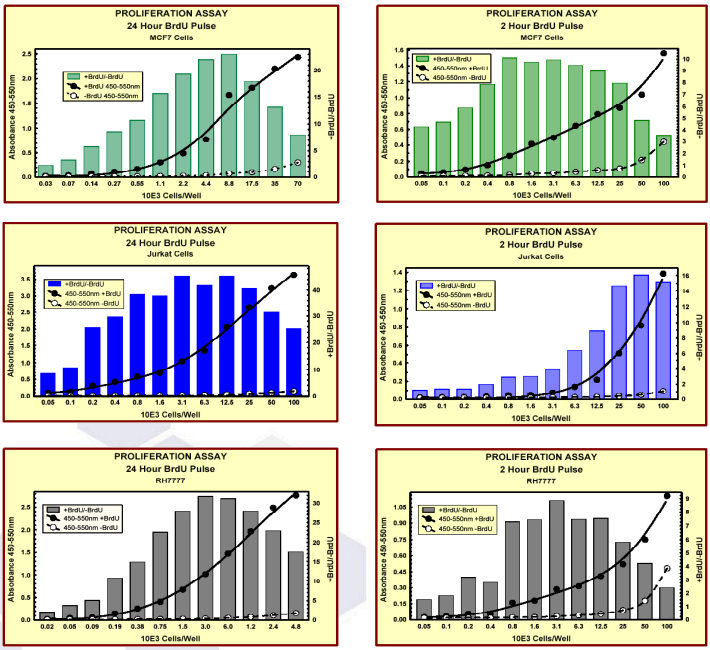

FIGURE 1.

Exalpha's Frontier™ BrdU cell proliferation assay, detection of variable numbers of Jurkat (non-adherent) or MCF7 or RH7777 (adherent) cells per well with 2 or 24 hour pulse with BrdU. Y axis - left, OD 450-550 nm. Y axis-right, signal -to-noise ratio.

Ordering Information

Catalog Number	Size
X1327K1	200 Tests
X1327K2	1000 Tests
X1327K3	5000 Tests

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