BrdU Cell Proliferation Assay Kit

Cat #: orb109970 & orb613770 (manual)

200 T/ 1000T

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

Product name: BrdU Cell Proliferation Assay Kit

Catalog number: orb109970 (200 T) & orb613770 (1000 T)

Species Reactivity: Ubiquitous

Intended Use

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

Storage of Kit Components

BrdU Cell Proliferation Assay Kit components are shipped on cold pack. Upon receipt, store kit at -20°C in a non-frost-free freezer. For long term storage, it is recommended that you aliquot and freeze the Prediluted Anti-BrdU Detector Antibody (Component 3) and 2000x Peroxidase Goat anti-Mouse IgG (Component 5) at -20 °C. Thirty (30) minutes prior to the use of each component, thaw component. Remove the Fixative/Denaturing Solution (Component 2) and place at room temperature for at least 4 hours prior to use. The Fixative/Denaturing Solution may contain slight precipitation and its color may vary between clear to light yellow. Return the Prediluted Anti-BrdU Detector Antibody (Component 3) and 2000x Peroxidase Goat anti-Mouse IgG (Component 5) to -20°C immediately after use. All other components may be stored at 4-8°C immediately after use. Special care should be taken to keep the Prediluted Anti-BrdU Detector Antibody (Component 3) and 2000x Peroxidase Goat anti-Mouse IgG (Component 5) cold by pulling out the number of aliquots needed for the test, keeping them on ice, and leaving the remaining aliquots at -20°C.

Background

The BrdU Cell Proliferation Assay Kit is a non-isotopic enzyme immunoassay for the quantification of DNA synthesis and cell proliferation.

Evaluation of cell cycle progression is essential for 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 has been demonstrated by numerous investigators. 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.

BrdU Cell Proliferation Assay Kit involves incorporation of BrdU Reagent into cells cultured in microtiter plates using the cell layer as the solid phase. The resultant assay is, 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

BrdU Cell Proliferation Assay Kit involves incorporation of BrdU Reagent into cells cultured in microtiter plates using the cell layer as the solid phase.

During the final 2 to 24 hours of culture 1X BrdU Reagent is added to wells of the microtiter plate. BrdU Reagent 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 Fixative/Denaturing Solution. Prediluted Anti-BrdU Detector Antibody is pipetted into the wells and allowed to incubate for one hour, during which time it binds to any incorporated BrdU Reagent.

Unbound Prediluted Anti-BrdU Detector Antibody is washed away and 1x Peroxidase Goat anti-Mouse IgG is added, which binds to the Prediluted Anti-BrdU Detector Antibody.

The 1x Peroxidase Goat anti-Mouse IgG catalyzes the conversion of the TMB Substrate (chromogenic substrate tetra-methylbenzidine) from a colorless solution to a blue solution (or yellow after the addition of ELISA Stop Solution), the intensity of which is proportional to the amount of incorporated BrdU Reagent in the cells. The colored reaction product is quantified using a spectrophotometer.

Materials Provided

The BrdU Cell Proliferation Assay is provided in 200, 1000 and 5000 test size. Volumes listed below are for the 200 test kit.

1. 500x BrdU Reagent: 15 µl.
2. Fixative/Denaturing Solution (Solution may contain slight precipitation and its color may vary between clear to light yellow.): 2 X 20 ml.
3. Prediluted Anti-BrdU Detector Antibody: 20 ml.
4. ELISA Stop Solution: 25 ml of 2.5N sulfuric acid.
5. 2000x Peroxidase Goat anti-Mouse IgG: 15 µl.
6. ELISA Conjugate Diluent: 25 ml Buffer for dilution of Conjugate.
7. TMB Substrate: 25 ml, Ready to use tetramethylbenzidine solution.
8. 50X Tris/Tween Plate Wash Concentrate: 90 ml concentrated solution of buffered Tris and surfactant.

Materials Required but Not Provided

1. 2-20 µl, 20-200 µl and 200-1000 µl precision pipettors with disposable tips
2. Wash bottle or multichannel dispenser for washing
3. 2000 ml graduated cylinder
4. PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4-7H2O, 1.4 mM KH2PO4)

5. Deionized or distilled H2O
6. 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.
7. Tissue culture microtiter plate (96 well culture dish)
8. Sterile reagent troughs
9. Micro syringe filter (0.2 µm)
10. Syringe

Summary Protocol

1. Cell Plating

1a. No Test Reagent/Drug

- Seed cells at 1-2 x 10^5 cells/ml, 100 µl/well.

1b. With Test Reagent/Drug

- Seed cells at 0.5-4 x 10^5 cells/ml, 100 µl/well.
- Add 100 µl/well, 2X concentration desired.

2. Addition of BrdU Reagent

- Dilute 500X BrdU Reagent in cell culture media, add 20 µl/well (be sure to include a No BrdU control).
- Incubate 2-24 hours.

3. Fix and Denature

3a. Adherent and Suspension Cells No-Spin Procedure

- Aspirate (or flick) the media from the cell wells.
- Add 200 µl/well Fixative/Denaturing Solution.
- Incubate 30 minutes at Room Temp.
- Aspirate the Fixative/Denaturing Solution and blot the plates dry.

3b. Suspension Cells Spin Procedure

- Spin the plates for 5 minutes at 1000 rpm.
- Aspirate media, add 200 µl/well Fixative/Denaturing Solution.
- Incubate for 30 minutes, room temp.
- Aspirate the Fixative/Denaturing Solution and blot the plates dry.

4. Wash Step

- Dilute the 50X Tris/Tween Plate Wash Concentrate in distilled water.
- Wash X3 with 1X Tris/Tween Plate Wash and blot dry.

5. Detector Antibody Addition

- Add 100 µl/well of Prediluted Anti-BrdU Detector Antibody.
- Incubate 1 hour at room temp.

6. Wash Step

- Wash X3 with 1X Tris/Tween Plate Wash and blot dry.

7. Conjugate Addition

- Dilute the 2000x Peroxidase Goat anti-Mouse IgG in ELISA Conjugate Diluent.
- Add 100 µl/well.
- Incubate for 30 minutes at room temperature.
- 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.

8. Development

- Add 100 µl/well TMB Substrate.
- Incubate for 30 minutes at room temperature in the dark.

9. Stop

- Add 100 µl of ELISA Stop Solution to every well.

10. Read

- Read the at 450/550 nm.

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 recommended to insure validity of the experiment.

- Blank: Add only tissue culture media (no cells).
- Background: Cells are present in the wells but no BrdU Reagent is added.

1. Cell Plating with and without Test Reagent(s)

1a. 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).

1b. Addition of Test Reagent (if applicable)

The test reagent can be a cell proliferation enhancer or alternatively, can induce growth inhibition or arrest. The test reagent is prepared at 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 Reagent addition (see step 2 below) will occur 2-24 hours prior to the end of the test reagent incubation.

2. Addition of BrdU

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

3. Fix and Denature Step and Storage of Fixed Plates

For detection of the BrdU Reagent by the Prediluted Anti- BrdU Detector Antibody, it is necessary to fix the cells and denature the DNA using a solution provided in this kit

(Fixative/Denaturing Solution). There is no need to spin the cells prior to addition of the Fixative/Denaturing Solution.

However, if suspension cells are being used, better precision is obtained if the cell plates are spun in a centrifuge prior to the fixative/denaturing step. Plates may be fixed (see steps 3a&b) and stored at 4°C 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.

3a. Adherent and Suspension Cells (No-Spin Fixative/Denaturing 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 Fixative/Denaturing Solution and incubate at room temperature for 30 minutes. Aspirate the Fixative/Denaturing Solution and blot the plate dry. Note: Fixed plates can be stored for up to 1 month at 4°C 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 Fixative/Denaturing Solution should be left in the wells).

3b. Suspension Cells (Spin Fixative/Denaturing 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 Fixative/Denaturing Solution. Incubate for 30 minutes at room temperature. Aspirate the Fixative/Denaturing Solution and blot the plates dry. The assay can be run immediately or plates may be stored for future use (see note above).

4. Wash Step

Dilute the 50X Tris/Tween Plate Wash Concentrate 1:50 by adding 40 ml 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 1X Tris/Tween Plate Wash. Wash

the plate three times with 1X Tris/Tween Plate Wash prior to adding Prediluted Anti-BrdU Detector Antibody. Aspirate the wash solution after the final wash and blot dry on paper towels.

5. Addition of Prediluted Anti-BrdU Detector Antibody

The Prediluted Anti-BrdU Detector Antibody is provided ready-to-use. Add 100 µl/well and incubate for 1 hour at room temperature.

6. Wash Step

Wash as in Step 4 above.

7. Preparation and Addition of the 2000x Peroxidase Goat Anti-Mouse IgG

The 2000x Peroxidase Goat Anti-Mouse IgG is provided as a concentrated stock solution. Dilute the Conjugate 1:2000 by adding 6 µl to 12 ml of ELISA 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.

8. Wash Step and Final Water Wash

Wash as in Step 4 above. Perform a final water wash by flooding the entire plate with distilled water. Pat dry on absorbent paper towels.

9. Development: Addition of TMB Substrate

Pipette 100 µl/well of the TMB 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 Reagent incorporation in the proliferating cells.

10. Addition of ELISA Stop Solution

Stop the reaction by pipetting 100 µl of ELISA Stop Solution provided to every well. The color of positive wells will change from blue to bright yellow.

11. Reading of the Plate

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).

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 Reagent for 24 hours and incorporated BrdU was detected with the BrdU Cell Proliferation Assay Kit. 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.

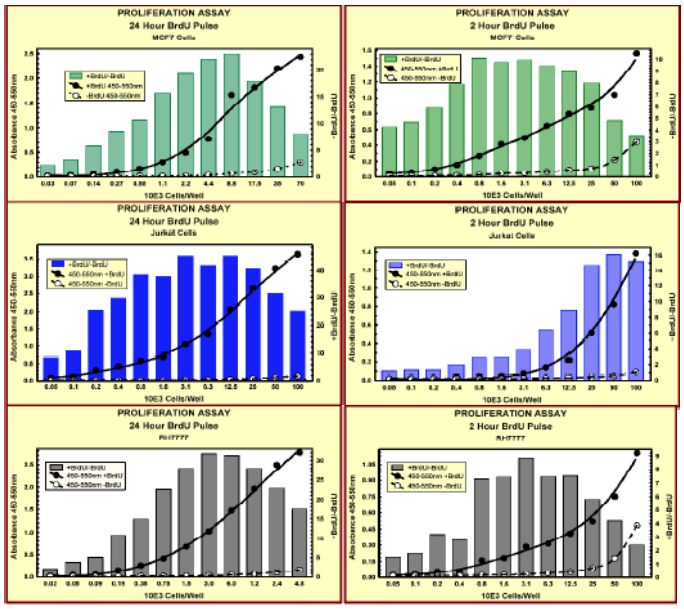

Figure 1.

BrdU Cell Proliferation Assay Kit, 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.