BrdU Chemiluminescent Cell Proliferation Assay Kit Instruction Manual

Features

- Fix/store and assay later
- Very Low Background
- Non-radioactive
- 2-hour protocol
- Best Signal/Noise (S/N) Ratio Available
- Chemiluminescent format
- HTS format compatible

Ordering Information

Catalog Number
X1623K

Size
200 and 1000 Tests

Format
ELISA Kit

Species Reactivity
Ubiquitous

Company Information
Exalpha Biologicals, Inc.
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Other Kits & Reagents Available from Exalpha Biologicals

DNA Fragmentation Detection Kit
X2044K1 (30 Tests)
X2044K2 (60 Tests)

BrdU Cell Proliferation Assay Kit
X1327K1 (200 Tests)
X1327K2 (1000 Tests)

BrdU Chemiluminescent Cell Proliferation Assay Kit
X1623K1 (200 Tests)
X1623K2 (1000 Tests)

BrdU Immunohistochemistry Kit
X1545K.1 (50 Sections)

BrdU Reagent for In Vivo Injection
X2834 (5 x 5 mg)

BrdU Unstained Control Slides
X2743 (5 slides)

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Intended Use

The Exalpha Biologicals, Inc. BrdU Chemiluminescent Cell Proliferation Assay Kit 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

Exalpha's BrdU Chemiluminescent 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 until the expiration date of the kit. 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 Chemiluminescent 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 [^{3}H] thymidine incorporation as cells enter S phase has long been the traditional method for the detection of cell proliferation. Subsequent quantification of [^{3}H] 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 [^{3}H] thymidine uptake has been demonstrated by numerous investigators. In these methods bromodeoxyuridine (BrdU), a thymidine analog, replaces [^{3}H] 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 Chemiluminescent 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

Exalpha Biologicals BrdU Chemiluminescent 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 oxidation of diacylhydrazides. The reaction product, in its excited state, decays yielding light in the process. This reaction has a large dynamic range spanning many logs of relative luminescence. The intensity of the luminescence is proportional to the amount of incorporated BrdU Reagent in the cells. The reaction is quantified using a luminometer and is plotted as relative light units per second (RLU/s).

Materials Provided

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

1. 500x BrdU Reagent
   (Part#J0012): 15 µl.

2. Fixative/Denaturing Solution (*Solution may contain slight precipitation and its color may vary between clear to light yellow.*)
   (Part #J0013): 2 X 20 ml.
3. Prediluted Anti-BrdU Detector Antibody
   (Part #J0014): 20 ml.
4. Chemiluminescent Reaction Buffer
   (Part #J0018): 24 ml reaction buffer for preparation of Chemiluminescent Substrate.
5. 2000x Peroxidase Goat anti-Mouse IgG
   (Part#J0016): 15 µl.
6. ELISA Conjugate Diluent
   (Part#J0004): 25 ml Buffer for dilution of Conjugate.
7. Chemiluminescent Substrate
   (Part #J0017): 800 µl.
8. 50X Tris/Tween Plate Wash Concentrate
   (Part#J0007): 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 H_2O
6. Luminometer
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.
8. 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.
9. Development
   • Prepare Chemiluminescent Substrate according to the instructions in the Detailed Protocol below.
   • Add 100 µl/well of prepared Chemiluminescent Substrate.
10. Read
    • Read immediately (reaction may be read for up to 30 minutes)

IMPORTANT NOTE: For best results, read plate immediately after substrate addition.

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 ensure 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 10^5 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 3 a&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. Addition of Chemiluminescent Substrate

NOTE: Prepare only enough Chemiluminescent Substrate to use immediately. Prepared Chemiluminescent Substrate can not be stored.

For 100 tests (One 96 well plate):
Prepare Chemiluminescent Substrate by adding 12 ml of Chemiluminescent Reaction Buffer to 400 µl of Chemiluminescent Substrate then mix. Immediately pipette 100 µl/well prepared Chemiluminescent Substrate and incubate for 5-10 minutes. Do not allow prepared Chemiluminescent Substrate to go unused for any length of time after mixing. Wells should be read immediately for best results. Well can be read for up to 30 minutes after addition of Chemiluminescent Substrate.

For 200 tests (Two 96 well plates):
Prepare Chemiluminescent Substrate by adding 24 ml of Chemiluminescent Reaction Buffer to 800 µl of Chemiluminescent Substrate then mix. Immediately pipette 100 µl/well prepared Chemiluminescent Substrate and incubate for 5-10 minutes. Do not allow prepared Chemiluminescent Substrate to go unused for any length of time after mixing. Wells should be read immediately for best results. Wells can be read for up to 30 minutes after addition of Chemiluminescent Substrate.

10. Reading of the Plate

Read the plate immediately using a luminometer (0.2 - 1 second integration time) or other alternative method. Plates can be read up to 30 minutes without significant degradation of signal after addition of Chemiluminescent Substrate, but it is strongly recommended to read immediately.

Ordering information

Catalog Number Size
X1623K1 200 Tests
X1623K2 1000 Tests

Contact Information

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