BrdU Cell Proliferation Assay
200 Tests

Cat. No. 2750

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Evaluation of cell cycle progression is essential for investigations in many scientific fields. Measurement of [3H] thymidine incorporation as cells enter S phase has been a traditional method for detection of cell proliferation. Subsequent quantification of [3H] thymidine is performed by scintillation counting of 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 researchers. 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 synthesizing DNA.

Test Principle

With Millipore©'s BrdU Cell Proliferation Assay Kit, mouse monoclonal antibody is used to detect the BrdU in a sample. After addition of goat anti-mouse IgG-peroxidase conjugated secondary antibody, substrate and stop solution, the amount of BrdU is determined. The higher the OD, the higher the BrdU concentration in the sample.

Application

The Millipore© BrdU Cell Proliferation Assay Kit is a non-isotopic assay for the in vitro quantitative detection of newly synthesized DNA of actively proliferating cells.

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Kit Components

1. BrdU Reagent: (Part No. 2750a) One 15 µL vial of a 500X solution of BrdU.
2. Fixing Solution: (Part No. 2750b) One 40 mL bottle.
3. Prediluted BrdU Detection Antibody: (Part No. 2750c) One 20 mL vial of an anti-BrdU in solution.
4. Goat anti-Mouse IgG, Peroxidase labeled: (Part No. 2750e) One 15 µL vial of a 2000X solution.
5. Conjugate Diluent: (Part No. 2750f) One 25 mL bottle.
6. Substrate: (Part No. 2750g) One 25 mL bottle, ready to use TMB.
7. 50X Plate Wash Concentrate: (Part No. 2750h) One 90 mL bottle of buffered Tris with surfactant.
8. Stop Solution: (Part No. 2750d): One 25 mL bottle of 2.5N sulfuric acid solution.

Materials Not Supplied

1. Pipettors & tips capable of accurately measuring 2-1000 µL
2. Multi-channel or repeating pipettes
3. Wash bottle or multichannel dispenser for washing
4. 2000 mL graduated cylinder
5. PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4-$7H_2O$, 1.4 mM KH_2PO_4)
6. Deionized or distilled H_2O.
7. 96-well microplate reader with 450-540 nm or 450-595 nm dual wavelength or 450 nm single wavelength filter.
8. Tissue culture microplate (96 well culture dish)
9. Sterile reagent troughs
10. Micro syringe filter (0.2 µm)
11. Syringe

Precautions and Recommendations

1. Do not expose the reagents to excessive light.
2. Wear disposable gloves and eye protection.
3. Do not use kit beyond expiration date.
4. Do not mix reagents from different kits.
5. Do not mouth pipette or ingest any of the reagent.
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 reagents.
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 wounds, or breathe aerosols. Dispose of samples properly.

Storage

Maintain the kit at 2º to 8ºC under sterile conditions for up to 6 months after date of receipt.
Before first use: remove the Fixative Solution and place at room temperature for at least 4 hours prior to use. Precipitates that may occur during cold storage should go back into solution.

Staining Protocol (for 8-well chamber slides)

1. **Controls**

   Two types of controls are recommended to insure the validity of the experiments.
   a. Blank: add only tissue culture supernatant (no cells)
   b. Background: cells are present in the wells but not have any added BrdU reagent.

2. **Cell Plating**

   Seed cells using a sterile 96-well tissue culture plate, cells are plated at 2 x 10^5 cell/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 did not receive cells (Blank) and wells, which contain cells but will not receive any BrdU reagent (Background).

3. **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 4 below) will occur 2-24 hours prior to the end of the test reagent incubation.

4. **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 signal to noise ratios are obtained when longer BrdU labeling times are used. Dilute 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 (Background control). Incubate the assay 2-24 hours.

5. **Fix and Denature Step and Storage of 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 the Fixing Solution. There is no need to spin the cells prior to the 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 6-7) and stored at 2º to 8ºC for assay at a later time. Place dried plates in a sealed dry plastic bag, zip-lock or heat sealed bags are suitable for this purpose. Plates are stable for at least one month when stored properly.

6. **Adherent and Suspension Cells (No-Spin Procedure)**

Aspirate the media from the cell wells (this can be done mechanically or the plates can be inverted over an appropriate reservoir and blotted on absorbent paper towels). Add 200 µL/well of the 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 one month at 2º to 8ºC if dried plates are sealed in a dry plastic bag (zip-lock or heat sealed bag). If storing your plates for future use, make sure that the plates are blotted well and are very dry.
Important: No Fixing Solution should be left in the wells).

7. **Suspension Cells (Spin Fix Procedure)**

Spin the plates in the centrifuge (using appropriate centrifuge microplate holders) for 5 minutes at 1000 rpm. Aspirate the media and add 200 µL/well of the Fixing Solution. Incubate at room temperature for 30 minutes. Aspirate the Fixing Solution and blot the plate dry.
The assay can be run immediately or plates may be stored for future use (see above).
Important: No Fixing Solution should be left in the wells).

8. **Wash Step**

Dilute the 50X Wash Buffer 1:50 by adding 40 mLs to 1.96 liters of distilled water. A microplate 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. Aspirate the wash solution after the final wash and blot dry on paper towels.

9. **Detector Antibody**

The anti-BrdU monoclonal is provided at as a prediluted solution. Add 100 µL/well of diluted antibody and incubate for 1 hour at room temperature.

10. **Wash Step**

Wash as in Step 8 above.

11. **Goat anti-Mouse IgG, Peroxidase Conjugate**

The Goat anti-Mouse IgG, Peroxidase Conjugate is provided as a 2000X concentrate. Dilute the Conjugate 1:2000 by adding 6 µL to 12 mLs with Conjugate Dilute provided. Once diluted this solution must be filtered using a 0.22 µm syringe filter. This lowers the background and improved the precision of the assay. Pipette 100 µL/well and incubate for 30 minutes at room temperature.

12. **Wash Step and Final Water Wash**

Wash as in Step 8 above. Perform a final wash by flooding the entire plate with distilled water. Pat dry on absorbent paper towels.

13. **Addition of Substrate**

Pipette 100 µL/well of 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.

14. **Addition of Stop Solution and Reading of the Plate**

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

Calculation of Results

The higher the OD reading the higher the BrdU concentration in the sample.

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