



BrdU Immunohistochemistry Kit

50 Tests

Cat. No. 2760

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

CHEMICON[®]'s BrdU Immunohistochemistry Kit is a non-isotopic immunohistochemistry staining kit for the detection and localization of BrdU incorporated into newly synthesized DNA of proliferating cells. Visual staining of the cells is achieved by using a biotinylated monoclonal antibody to BrdU followed by streptavidin-HRP conjugate and DAB substrate.

Application

The CHEMICON[®] Kit is designed for *in vitro* detection of BrdU in proliferating cells. The material in this kit is sufficient to run 50 slides. The average test area is defined as a circle around the tissue with an approximate diameter of 2 centimeters.

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Kit Materials

1. 4X Trypsin Enzyme Concentrate*: (Part No. 2760a) One 3 mL bottle.
2. Trypsin Dilution Solution*: (Part No. 2760b) One 12 mL bottle.
3. Denaturing Solution: (Part No. 2760c) One 6 mL bottle.
4. Blocking Buffer: (Part No. 2760d) One 6 mL bottle.
5. Detector Antibody (Biotinylated and ready-to-use): (Part No. 2760e) One 6 mL bottle.
6. Streptavidin-HRP Conjugate (ready-to-use): (Part No. 2760f) One 6 mL bottle.
7. Substrate Reaction Buffer: (Part No. 2760g) One 6 mL bottle.
8. DAB Concentrate: (Part No. 2760h) One 0.3 mL.
9. Hematoxylin Counterstain: (Part No. 2760i) One 6 mL bottle.
10. Mounting Media: (Part No. 2760j) One 6 mL bottle.
11. 5 Control Slides: Intestinal tissue from mouse injected with BrdU.
1 Positively stained and 4 unstained (Part No. 2760k). The unstained slides are paraffin embedded.

**Trypsin is only required if you are using formalin fixed tissues. If the tissues are fixed in alcohol, trypsin digestion is not necessary.*

Materials Not Supplied

1. Quenching Solution: Hydrogen peroxide (30%) solution for quenching endogenous peroxidase activity.
2. Phosphate buffered saline (PBS) solution.
3. Distilled water.
4. Ethyl alcohol
5. Xylene
6. Coverslips
7. Bromodeoxyuridine (BrdU)
8. Methanol

Preparation of Slides

Paraffin-embedded tissue sections

1. Sample animals are labeled with BrdU.
2. Animals are sacrificed by inhalation of isofluorane and perfused with PBS followed by 4% buffered formalin.
3. Target tissue is removed and immersed in 4% buffered formalin overnight.
4. Tissue is then dehydrated and embedded in paraffin
 - a. PBS for 10 minutes
 - b. 70% EtOH for 1 hour
 - c. 85% EtOH for 1 hour
 - d. 95% EtOH for 30 minutes
 - e. 100% EtOH for 15 minutes (2 times)
 - f. Xylene for 15 minutes (2 times)
 - g. 1:1 Xylene and Paraffin for 45 minutes
 - h. Paraffin for 30 minutes (4 times)
5. 5 micron sections are cut from the paraffin blocks and placed on slides.
6. Slides remain on a 37°C heating tray overnight and are stored at 2° to 8°C.

Cultured Cells and Cell Suspensions

Preparation of cells

A. Cell Flasks

1. Using sterile tissue culture techniques, culture cells with 10 μ M of BrdU for 2-24 hours at 37°C.
2. Remove the media containing the BrdU label and wash 2 times with PBS.
3. Using a cytospin, centrifuge 100 μ L of cell at 1×10^6 cells/mL onto suitable slides and allow to air dry.

B. Cell on Chamber Slides (Adherent cells only)

1. Using sterile tissue culture techniques, culture cells in chambers with 10 μ M of BrdU for 2-24 hours at 37°C.
2. Remove the labeling media and wash 2 times with PBS.
3. Fix cells with 70% ethanol or other suitable fixative for 30 minutes.
4. Wash twice with PBS.

Proceed with Staining Protocol

Storage of Kit and Kit Components

Maintain the unopened kit at -20°C for up to 6 months after date of receipt. After opening the kit it may be stored at 2° to 8°C for 4-5 days. For long-term storage it is recommended that you aliquot each component and freeze at -20°C for up to 3 months after opening. It is recommended that you wear latex or rubber gloves when running this kit, especially avoid contact with the DAB reagent.

Staining Protocol

Deparaffinization (For Paraffin-Embedded Tissues Only)

Note #1: If you are not using paraffin embedded tissue then skip to Step 1 below.

Note #2: The unstained control slides are paraffin embedded and need to be deparaffinized.

Deparaffinization involves incubated of the slides in xylene followed by a graded alcohol series as follows:

Xylene	5 minutes, then change to a new coplin jar containing Xylene
Xylene	5 minutes
100% ethyl alcohol	5 minutes
90% ethyl alcohol	3 minutes
80% ethyl alcohol	3 minutes
70% ethyl alcohol	3 minutes
PBS	3 minutes

Staining

1. Immerse slides into a coplin jar or other container filled with Quenching Solution (Dilute 30% hydrogen peroxide* 1:10 in methanol) for 10 minutes. Wash with 1X PBS for 2 minutes.
2. **For Paraffin Embedded Tissue Sections Only:** Add 2 or more drops of 0.2% Trypsin Solution** (Add 1 drop of 4X Trypsin Enzyme Concentrate to 3 drops of Trypsin Dilution Solution and mix well) to each slide. Incubate at room temperature for 10 minutes, followed by a 3 minute rinse in distilled water.
3. Add 2 or more drops of Denaturing Solution to each slide and incubate at room temperature for 30 minutes. Wash twice with PBS, 2 minutes per wash.
4. Add 2 or more drops of Blocking Solution to each slide and incubate at room temperature for 10 minutes. Drain the solution by blotting on paper towels (Do Not Rinse).
5. Add 2 or more drops of the Detector Antibody to each section and incubate at room temperature for 60 minutes. Wash twice with PBS, 2 minutes per wash.

6. Add 2 or more drops of Streptavidin-HRP Conjugate to each section and incubate at room temperature for 10 minutes. Wash twice with PBS, 2 minutes per wash.
7. Add 1 μL of DAB concentrate for every 29 μL of Substrate Reaction Buffer (assume approximately 100 μL /slide). For 10 slides this works out to be 1 drop of DAB concentrate to 1 mL of the Substrate Reaction Buffer. Mix well and add 2 or more drops per slide and incubate at room temperature for 10 minutes. Wash with distilled water for 2 minutes.
8. Add 2 or more drops of Hematoxylin Counterstain per slide and incubate at room temperature for 1-5 minutes. Wash slides briefly with tap water. Incubate slides for 1 minute in PBS until color turns blue. Give a final 2 minute wash in distilled water.
9. Incubate slides in 90% ethanol for 30 seconds, 100% ethanol for 30 seconds and xylene for 30 seconds (2 times each). Add 1-2 drops of mounting media and coverslip.

* Hydrogen peroxide is not stable for long periods of time. Be sure that the reagent you are using has not expired.

** The concentration of trypsin used is very important. It may be necessary to titer the trypsin reagent for use in your system. Usually a final concentration of 0.02% to 0.2% is appropriate. Other methods for digesting the tissue to expose epitopes for antibody recognition may also be used.

Troubleshooting:

Poor positive staining or No positive staining with little or no background staining

1. Little or no BrdU labeling occurred in the tissues or cells prior to preparing the slides.
2. Detector Antibody or Streptavidin-HRP reagent was omitted or used in the wrong order.
3. Use a longer incubation time for the Detector Antibody.
4. Use a longer incubation time for the substrate (view slide with it is developing).
5. Since excessive counterstaining can compromise positive brown DAB staining, try using shorter hematoxylin counterstain incubation time.
6. DO NOT LET SLIDES DRY OUT; keep wet at all times during the staining procedure.
7. Insufficient blotting between blocking step and detector antibody step. This could dilute out the Detector Antibody component.
8. If tissue is formalin fixed and digestion of tissue is necessary, the trypsin component may need titering.
9. Use fresh xylene solution as a solution that has been used many times will contain residual paraffin and may interfere with the staining.

High Background Staining

1. Reduce substrate incubation time.
2. Check to be sure that the substrate-DAB reagent was prepared correctly (the right ratio of DAB concentration to Substrate Reaction Buffer).
3. Reduce concentration of the Streptavidin-HRP component.
4. Increase the number and time of washes between steps.
5. Slides were incorrectly deparaffinized (use fresh reagents).

Warranty

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